# Defining Quercetin-, Caffeic acid- and Rosmarinic acid-mediated life extension in *C. elegans*: Bioassays and expression analyses

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*„Damit das Mögliche entsteht, muss das Unmögliche immer wieder versucht werden.“*

Hermann Hesse

*für meinen Sohn Dean*

# Contents

**Summary** **5**
**Zusammenfassung** **6**
**List of used Abbrevations** **7**
**1 Introduction** **11**
1.1 Background 11
1.2 Phytochemicals in plant-based food 11
1.2.1 Polyphenols 12
1.3 The model organism *Caenorhabditis elegans* 15
1.3.1 *C. elegans* in aging research 17
1.4 Aging 19
1.4.1 Why do we age? - Selected aging theories 20
1.4.2 Modulation of lifespan: caloric restriction and hormesis 26
1.5 Goals of this study 30
**2 Material and methods** **34**
2.1 Strains and culture conditions 34
2.2 Life table parameters and functional investigations 34
2.2.1 Lifespan assays 34
2.2.2 Reproduction 35
2.2.3 Growth alterations 35
2.2.4 Pharyngeal pumping rate 36
2.2.5 Stress resistance 36
2.2.6 Attraction assay 36
2.2.7 Bacterial growth assay 36
2.2.8 Fluorescence measurements 36
2.2.9 Total Antioxidative Capacity (TAC) 37
2.2.10 Data interpretation and statistical analysis 38
2.3 Molecularbiological experiments 38
2.3.1 Expression levels of heat shock protein genes (*hsp*s) quanified by reverse transcriptase-PCR (qRT-PCR) 38
2.3.2 DNA-microarray analyses 39
**3 Results** **41**
3.1 Possible artifacts: antimicrobial properties and transgenerational effects 41
3.1.1 Q and RA diminish the bacterial growth of *E. coli* 41
3.1.2 Growing on heat-killed *E. coli* does not prevent life extension 41
3.1.3 Transgenerational effects of Q treatment are negligible 42
3.2 Hormetic working modes 44
3.2.1 PPs display concentration-dependent lifespan extension 44
3.2.2 Induction of *hsp* expression levels is variable 46
3.3 Direct and indirect CR effects 47
3.3.1 PPs enhance the pharyngeal pumping rate throughout the life 47
3.3.2 *C. elegans* are not attracted nor repulsed by PPs 47
3.3.3 *sir-2.1* lifespan cannot be extended by CA and RA 47

3.4 Re-allocation of resources 48
3.4.1 Reproduction: unchanged broodsize but delays in timing 48
3.4.2 PPs influence the growth of young larvae and adults 49
3.4.3 PPs change intestinal fat deposition 50
3.5 Antioxidative properties 52
3.5.1 PP-distinct total antioxidative capacitiy (TAC) *in vivo* and *vitro* 52
3.5.2 Q prevents, RA enhances paraquat-induced matricidal 53
3.5.3 All three PPs prolong the lifespan of *mev-1* mutants 54
3.5.4 Intestinal lipofuscin fluorescence is decreased due to Q and CA 56
3.6 Genetic background: mutant lifespan and thermotolerance assays 57
3.6.1 Genetic players of Q mediated longevity 57
3.6.2 Genetic players of CA and RA mediated longevity 61
3.7 Global transcriptional profiling: DNA-microarray results from Q treated nematodes 65
3.7.1 Transcriptional profiles 65
3.7.2 Gene Ontology (GO) Analysis 66
3.7.3 Overrepresented KEGG-pathways 72
3.7.4 Selected gene-groups and –classes 77
3.7.5 Meta-analysis: comparison of global transcriptional profiles in $Q_{longevity}$ and selected datasets in the literature 79
**4 Discussion 96**
4.1 Does the test design produce artifacts? 96
4.2 Is longevity promoted via hormesis? 96
4.3 Do direct or indirect CR effects contribute to life extension? 97
4.4 Does disposable soma-like re-allocation of resources take place? 98
4.5 Are antioxidant properties *in vivo* responsible for longevity? 98
4.6 Which genetic players promote longevity and stress-resistance? 100
4.6.1 Genetic mechanisms of Q's action 101
4.6.2 Genetic mediators of CA's and RA's action: mutant lifespan assays 105
4.7 Do Q, CA and RA use common or exclusive mechanisms? 109
4.8 Is it possible to apply common aging theories to the results of this study? 111
**5 Conclusion 113**
5.1 What can be learned from this study? 113
5.1.1 General working scheme of PP action 113
5.1.2 Outlook and future directions 114
5.1.3 Critical review 116
**6 References 118**
**Appendix 138**
**Danksagung 183**
**List of Publications 184**
**Selbständigkeitserklärung 186**

## Summary

The mean life expectancy of humans has increased continuously over the last 200 years. Since longevity is of little value in the absence of health, it is a central request to prevent the increasing burden of age-related diseases. As shown by long-living populations in Okinawa and the Mediterranean, nutrition plays a prominent role in extending the healthspan. It is suggested that phytochemicals in plants, specifically the polyphenols (PPs), are important factors to support the overall well-being. However, the precise mechanisms that can explain, in full, the magnitude of impact remains elusive. It has still not been established whether certain PPs use common, overlapping or exclusive mechanisms, whether antioxidative properties, energy re-allocation effects or hormesis-based action modes are responsible and which genetic players are involved. This knowledge gap can only be plugged by *in vivo* model organism approaches that integrate lifespan assays with physiological, and genetic parameters following the ingestion of PPs.

In this study, three PPs with life-extending properties in *C. elegans* were identified: Quercetin (Q), Caffeic acid (CA) and Rosmarinic acid (RA). After ruling out that the longevity effect is provoked by experimental artifacts, like antibacterial properties of the PPs against the *E. coli* feeding strain or direct caloric restriction (CR) effects due to gustatory repulsion, the underlying mechanisms were systematically studied by a broad spectrum of functional and genetic investigations. For all three compounds, life extension was characterized by hormetic concentration-response curves, but stress-response induction, a hallmark of hormetin action, was restricted to Q and RA, at least when assessed at the level of gene expression of heat shock proteins. A reallocation of resources in a disposable soma-like pattern could be shown for all three PPs, because the exposure to Q, CA and RA resulted in variations in body size, altered lipid-metabolism and a tendency towards a delay in reproductive timing. However, not all physiological parameters were affected by PPs, since, for example, the total number of offspring was unaltered. While direct CR effects arising from reduced food uptake could be rejected, an indirect CR effect cannot be excluded for CA and RA, as these PPs failed to provoke longevity in *sir-2.1* mutants. Furthermore, the *in vitro* versus *in vivo* antioxidative properties were evaluated, through lifespan assays with endogenous ROS stressed *mev-1* mutants, an oxidative stress test with paraquat, a total antioxidative capacity (TAC) assay, and by determining the intestinal lipofuscin fluorescence, a marker for the amount of oxidative damaged macromolecules. While all three PPs could prolong *mev-1* lifespan, only Q and CA were shown to increase the TAC *in vivo* and reduce oxidative damage in the nematodes. To define the genetic pathways of PP action, lifespan and thermotolerance assays were performed in mutant animals devoid of aging-relevant genetic players. These experiments revealed that the health gaining properties of CA and RA both rely on *osr-1*, *sek-1*, *sir-2.1* and *unc-43*, plus *daf-16* in the case of CA. The mechanisms of Q action are partly distinct and were analyzed in more detail by integrating own mutant lifespan assays and DNA-microarray studies with an extensive meta-analysis of published gene expression profiles obtained under aging-relevant conditions. Quercetin is proposed to act through a complex interplay of conserved genetic pathways, for example Insulin-like signaling (ILS), TGF-beta signaling, p38 MAPK, CaMKII, and possibly also due to germline and somatic gonad signaling.

Taken together, hormesis, *in vivo* antioxidative/prooxidative properties, modulation of genetic players, as well as the re-allocation of resources all contribute (to some extent and dependent on the polyphenol) to life extension.

# Zusammenfassung

Die mittlere Lebenserwartung des Menschen ist über die letzten 200 Jahre kontinuierlich gestiegen. Da Langlebigkeit ohne Gesundheit wenig Wert besitzt, ist es ein zentrales Anliegen, das Auftreten altersbedingter Krankheiten zu mindern. Wie durch langlebige Populationen in Okinawa und dem mediterranen Raum gezeigt, spielt die Ernährung dabei eine wichtige Rolle. Besonders pflanzliche Phytochemikalien, im speziellen Polyphenole (PPs), sollen erheblich an der Gesundheitsförderung mitwirken. Die exakten Mechanismen jedoch, welche die Wirkvielfalt erklären könnten, sind nicht im Detail bekannt. Weder ist ermittelt, ob bestimmten PPs dieselben, überlappende oder jeweils eigene Wirk-Wege zugrundeliegen, ob antioxidative *in vivo* Eigenschaften, Energie-Umverteilungs- oder Hormesis-basierte Effekte verantwortlich sind, noch welche Gene daran beteiligt sind. Diese Fragen können nur durch *in vivo* Studien an Modelorganismen beantwortet werden, die sowohl die Lebensdauer, sowie physiologische und genetische Parameter einschließen.

In dieser Studie wurden drei PPs mit lebensverlängernden Eigenschaften in *C. elegans* identifiziert: Quercetin (Q), Kaffeesäure (CA) und Rosmarinsäure (RA). Nachdem experimentelle Artefakte als Grund für die Langlebigkeit ausgeschlossen waren (wie z. B. antibakterielle Wirkungen der PPs gegenüber den Futterbakterien oder direkte Kalorien-Restriktions (CR)- Effekte ausgelöst durch eine verminderte Futteraufnahme), wurden zugrundeliegende Mechanismen mit einem breiten Spektrum an funktionellen und genetischen Analysen systematisch untersucht. Für alle drei PPs wurden hormetische Konzentration-Wirkungs-Kurven gefunden, dennoch war die Hormetin-typische Aktivierung einer Stressantwort (gemessen als Geneexpressions-Level von Hitzeschock-Proteinen) auf Q und RA beschränkt. Eine Umverteilung von Ressourcen nach dem Prinzip der „Disposable Soma Theorie“ konnte anhand von Abweichungen in der Größe, verändertem Lipid-Metabolismus und verzögerter Reproduktion (bei gleichbleibender Anzahl der Nachkommen), für alle drei PPs gezeigt werden. Während direkte CR-Effekte ausgeschlossen wurden, ist dies nicht möglich für durch CA und RA ausgelöste indirekte CR-Effekte, da beide die Lebensspanne von *sir-2.1* Mutanten nicht verlängern konnten. Darüberhinaus wurden *in vitro* und *in vivo* antioxidative Eigenschaften mittels Lebensdauertests mit endogen gestressten *mev-1* Mutanten, einem oxidativen Stresstest mit Paraquat, einer Messung der Totalen Antioxidativen Kapazität (TAC) und über die Bestimmung der intestinalen Lipofuscin-Fluoreszenz als Marker für die Menge an oxidativ geschädigten Makromolekülen bestimmt. Alle drei PPs verlängerten die Lebensspanne von *mev-1* Mutanten, jedoch wurde eine erhöhte TAC *in vivo* und eine reduzierte oxidative Schädigung, nur durch Q- und CA- Gabe erreicht. Die genetischen Wirkwege der PPs wurden durch Lebensdauer- und Thermotoleranztests mit in alters-relevanten Genen mutierten Nematoden definiert. Die gesundheitsfördernden Eigenschaften von CA und RA konnten so *osr-1*, *sek-1*, *sir-2.1* and *unc-43,* sowie *daf-16* im Falle von CA, zugeschrieben werden. Die Mechanismen von Q wurden in größerem Umfang, durch die Integration von durchgeführten Lebensdauertests und DNA-Microarray-Studien einerseits und einer umfassenden Meta-Analyse von veröffentlichten, alters-relevanten Genexpressions-Profilen andererseits, analysiert. Q wirkt vermutlich durch ein komplexes Zusammenspiel von konservierten genetischen Signalwegen, im Speziellen dem Insulin-ähnlichen (ILS), TGF-beta, p38 MAPK, CAMKII und möglicherweise auch über eine von der Keimbahn und somatischen Gonade ausgehenden Signalwirkung.

Zusammenfassend lässt sich sagen, dass sowohl *in vivo* antioxidative und prooxidative Eigenschaften, die Modulation auf Genebene, sowie eine Umverteilung von Ressourcen zu gewissen Teilen (abhängig vom PP) zur Lebensverlängerung beitragen.

# Abbrevations

| | |
|---|---|
| AA | Ascorbic acid |
| ACL | Antioxidative capacity of lipid-soluble substances |
| ACW | Antioxidative capacity of water-soluble substances |
| ADPKD | Autosomal dominant polycystic kidney disease |
| ANOVA | Analysis of Variance |
| BP | Biological Process |
| CA | Caffeic acid |
| CaMKII | $Ca^{2+}$/ Calmodulin-dependent kinase |
| CAT | Catalase |
| CC | Cellular Component |
| cDNA | Complementary DNA |
| CeMM | *C. elegans* Maintenance Medium |
| CR | Caloric Restriction |
| CVD | Cardio vascular disease |
| CYP | Cytochrome P450 |
| *cyp*s | Cytochrome P450 genes |
| d | Day |
| DAVID | Database for Annotation, Visualization and Integrated Discovery |
| DEGs | Differential regulated genes |
| DMSO | Dimethylsulfoxid |
| dsRNA | double stranded RNA |
| EGCG | Epigallocatechin-3-Gallat |
| F1 | $1^{st}$ filial generation |
| F2 | $2^{nd}$ filial generation |
| FDR | False discovery rate |
| FRTA | Free Radical theory of aging |
| GEPAS | Gene Expression Pattern Analysis Suite |
| GFP | Green-fluorescence protein |
| GO | Gene Ontology |
| GST | Gluthadion s-transferase |
| *gst*s | Gluthadion s-transferase genes |
| HSP | Heat shock protein |
| *hsp*s | Heat shock protein genes |
| IGF-1 | Insulin-growth factor 1 |
| ILS | Insulin-like Signaling |
| IP | InterPro |
| IVT | *In vitro* transcription |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| L1 | First larval stage |
| L2 | Second larval stage |
| L3 | Third larval stage |
| L4 | Fourth larval stage |
| LPS | Lipopolysaccharide |
| LROs | Lysosome related organells |
| MF | Molecular Function |
| *msp*s | major sperm protein |
| MTA | Mitochondrial theory of aging |
| MW | Molecular Weight |
| n | Sample size |
| NAD(P)H | Reduced form of nicotinamide adenine dinucleotide phosphate |
| NGM | Nematode growth medium |
| OD | Optical density |
| p | Statistical significance |
| -p | Without paraquat |
| +p | With paraquat |

| | |
|---|---|
| P0 | Parental generation |
| p38 MAPK | Protein 38 mitogen activated protein kinase |
| p53 | Tumor protein 53 |
| PCA | Principal Component Analysis |
| PI3K | Phosphoinositide 3-kinase |
| PIP2 | Phosphatidylinositol-3,4-diphosphate |
| $PIP_3$ | Phosphatidylinositol-3,4,5-triphosphate |
| PP | Polyphenol |
| PQ+ | Stable paraquat radical |
| Q | Quercetin |
| $Q_{all}$ | DEGs commonly regulated in 50, 100 and 200µM Q |
| $Q_{longevity}$ | DEGs commonly regulated in 100 and 200 µM Q |
| qRT-PCR | Real-time quantitative reverse transcriptase polymerase chain Reaction |
| RA | Rosmarinic acid |
| RF | Representation Factor |
| RNA | Ribonucleic acid |
| RNAi | RNA interference |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| SAM | Significance of microarray |
| SDR | Short-chain dehydrogenase/reductase |
| SEM | Standard error of the mean |
| SIR2 | Silent Information Regulator 2 |
| SOD | Superoxid dismutase |
| T | Trolox |
| TAC | Total Antioxidant Capacity |
| TGF-beta | Transforming Growth Factor-beta |
| TOR | Target of rapamycin |
| UGT | UDP glucuronosyltransferase |
| *ugt*s | UDP glucuronosyltransferase genes |
| UV | Ultra violet |
| vWF | Von Willebrand factor |

# 1 Introduction

## 1.1 Background

Since the beginning of time, humans have searched for the “Fountain of Youth”, as the desire to live forever is neither new nor particularly Western, but rather a universal dream (Minois, 1987). Barely a week goes by without the media bringing up new and plausible hopes that we are just a technical breakthrough away from eternal youth or at least the extension of lifespan.

Indeed, aging and the increase of life expectancy demonstrates one of the most impressive success stories in human history. In the last 200 years the mean life expectancy of humans has increased continuously such that in the last century lifespan has enhanced by approximately 2 years every decade and this trend is expected to persist (Dominguez et al., 2009). Initially, the reasons for increased life expectancy included improvements in sanitation, housing and education, which produced a decline in early and mid-life mortality due to the prevention and treatment of infectious diseases. The further increase in life expectancy in the second half of the past century referred predominantly to medical advances and resulted in a decline in late-life mortality (Kirkwood, 2008). Consequently, the proportion of people aged over 60 years is growing fast. However, longevity is of little value in the absence of quality of life. All industrialized countries face a growing prevalence of chronic age-related conditions. Hence, huge social interest in the civilized world exists, to extend the “health-span” of the individual. Beside caloric restriction, hormesis and genetic manipulations, which in fact could be shown to delay aging and death in model organisms (reviewed in Olsen et al., 2006), but with yet rare evidence for transferability to humans, a balanced nutrition and lifestyle remain today the simplest and only proven “Fountain of Youth” (Dominguez et al., 2009).

*Nutrition affects aging*: “We are what we eat.” (taken from Pérez-López et al., 2009). In this context, the popularity of the Mediterranean as well as the traditional Okinawan diet, which both share many of the same composition characteristics, has grown worldwide during the last 20 years, due to their link with greater longevity, higher numbers of centenarians and lower rates of cardiovascular disease, cancer and age-associated cognitive decline (Trichopoulou et al., 2005; Serra-Majem et al., 2006). People on a Mediterranean diet for example, had more than a 20% lower risk of dying during a 10-year period compared to those with a differing food composition, independent of their age, body weight and gender (Knoops et al., 2004). The health and longevity effect is in parts attributed to fruits and vegetables containing biologically active phytochemicals, particularly the antioxidant polyphenols (PPs) (Willcox et al., 2009), that are likely to interact in a number of ways to prevent disease and promote health (Liu, 2004).

## 1.2 Phytochemicals in plant-based food

Plant-based foods are prominent features of healthy dietary patterns. Despite all of the controversy surrounding the optimal components of a healthy diet, there is little disagreement among scientists regarding the importance of fruits and vegetables (including legumes, whole grains and nuts; National Academy of Sciences, 1982, 1989; Liu, 2003).

*Evidence and future directions:* So far results of numerous epidemiological studies and recent clinical trials provide consistent proof that diets rich in plants have significant protective effects and can reduce the risk of chronic disease (Block et al., 1992; Lock et al., 2005). Up to 2.635 million deaths per year are attributable to an inadequate intake of plant-based food. Increasing

individual fruit and vegetable consumption up to 600 g per day could reduce the total worldwide burden of cardiovascular disease (CVD) and various types of cancer considerably (Lock et al., 2005).

In addition to providing energy and essential micronutrients, fruits and vegetables contain thousands of biologically active phytochemicals that are likely to interact in a number of ways to prevent disease and promote health (Liu, 2004). While there is ample evidence to support the health benefits of plant-based food, evidence that these effects are due to specific nutrients or phytochemicals is limited and exact mechanisms need to be deciphered. Therefore, it is reasonable for scientists to precisely scrutinize phytochemical mixtures extracted from edibles and identify the bioactive compounds responsible for the health beneficial effects of plant-based foods. The driving force behind this approach is the hope to find the magic bullet to prevent chronic diseases and thereby provide a convenient long-lasting life. Hence, current great effort focus on the identification of molecules of herbal origin, which are able to significantly decelerate deleterious impacts of aging *in vivo* and on the study of underlying physiological and genetic mechanisms to finally find new targets for pharmacological interventions (Wu et al., 2002; Viswanathan et al., 2005; Brown et al., 2006; Wilson et al., 2006; Kampkötter et al., 2007a, b, 2008; Saul et al., 2008, 2009, 2010; Wiegant et al., 2008; Pietsch et al., 2009, 2011).

*Classification of phythochemicals:* So far approximately 5000 individual phytochemicals have been identified in fruits, vegetables, and grains, but a large percentage still remain unknown. Phytochemicals can be classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Supplementary Table 1 summarizes phytochemicals contained in food. Phenolics are among the best-studied phytochemicals (Liu, 2004) and possess one or more aromatic rings with one or more hydroxyl group and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins. In plants phenolics are products of secondary metabolism, providing functions in reproduction, growth and colour and acting as defence mechanisms against pathogens, parasites, and predators. In addition to these aboriginal roles in plants, phenolic compounds ingested with our diet may provide health benefits to the consumer, due to adaptation processes in our metazoan ancestors (Stevenson and Hurst, 2007).

### 1.2.1 Polyphenols

Most promising in the efficacy of reversing age-related declines and preventing CVD and cancer seem to be polyphenols (PPs, characterized by two or more phenolic hydroxyl groups), as evidenced by multiple *in vivo* studies (Joseph et al., 1999, 2005; Aggarwal et al., 2004; Neuhouser, 2004; Manach et al., 2005a; Roginsky et al., 2005; Hodgson and Croft, 2006; Wilson et al., 2006). The bioavailability and bioefficacy of certain PPs have already been proven for humans (Rechner et al., 2002; Nielsen et al., 2003; Manach et al., 2004, 2005b). Antioxidant properties were believed to be the reason for the denoted beneficial effects, however, recent indications point towards antioxidative-independent mechanisms (Akagawa et al., 2003; Nakagawa et al., 2004; Galati et al., 2006; Maeta et al., 2007). PPs have been shown to alter gene expression patterns and act via different signaling cascades, involved in the regulation of cell growth, inflammation and many other processes (Williams et al., 2004; Narayanan, 2006;

Wilson et al., 2006; Pietsch et al., 2009, 2011). Under certain circumstances PPs appear to act as prooxidants which induce cellular stress responses and thereby reduce sensitivity to oxidative stress. For detailed review see Stevenson and Hurst (2007).

## The flavonoid Quercetin

It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids (Liu, 2003). More than 4000 distinct flavonoids have been identified. They commonly have a generic structure consisting of two aromatic rings (A and B rings) linked by 3 carbons that are usually in an oxygenated heterocycle ring, or C ring. Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and isoflavonoids.

Quercetin (Q, Fig. 1 A) belongs to the subclass of flavonols and is an important dietary flavonoid that is present in herbal food like apples, broccoli, onions and many more herbal diets. Within the flavonoid family, Q is the most potent scavenger of ROS (Reactive oxygen species; Hanasaki et al., 1994; Cushnie and Lamb, 2005), RNS (Reactive nitrogen species; van Acker et al., 1995; Haenen and Bast, 1999) and peroxynitrite (Haenen et al., 1997; Heijnen et al., 2001). These antioxidative capacities of Q are attributed to the presence of two antioxidant pharmacophores within the molecule, i.e. the catechol group in the B ring and the OH group at position 3 of the AC ring (Heijnen et al., 2002). Q is also known to possess strong anti-inflammatory capacities, by inhibition of LPS induced cytokine production (Read, 1995; Manjeet and Ghosh, 1999; Orsolic et al., 2004; Geraets et al., 2007; Bureau et al., 2008). Beyond above mentioned traits Q also possesses anti-fibrotic (Lee et al., 2003), anti-coagulative (Bucki et al., 2003), anti-bacterial (Cushnie and Lamb, 2005), anti-atherogenic (de Whalley et al., 1990; Perez-Vizcaino et al., 2006), anti-hypertensive (Duarte et al., 2001; Perez-Vizcaino et al., 2006) and anti-proliferative properties (Kuo, 1996; Orsolic et al., 2004; Gulati et al., 2006). Additionally, Q is reported to directly modulate the gene expression of enzymes involved in biotransformation (Walle et al., 1995; Pacifici, 2004; Schwarz et al., 2005; Moon et al., 2006) and to inhibit cell proliferation by interacting with estrogen binding sites (Piantelli et al., 1995; Caltagirone et al., 1997). Q reduces oxidative DNA strand break formation and the decrease in apoptotic cell death by oxidative stimuli (Wätjen et al., 2005). Altogether, these studies indicate that Q may exert health-beneficial capacities through various damage modulating effects. Toxic effects of Q were only observed *in vitro*. These effects are most likely associated with the formation of possible toxic products (like ROS) by autoxidation and redox-cycling (Ochiai et al., 1984; Hodnick et al., 1986; Gaspar et al., 1994; Metodiewa et al., 1999; Yoshino et al., 1999). Genotoxic effects have been also reported, but are predominantly shown in bacteria (Vrijsen et al., 1990; Jurado et al., 1991; Rueff et al., 1995; Silva et al., 2000). However, most of the studies performed so far have involved the use of immortalized or cultured cell lines and are thus not easy to extrapolate to the whole animal, *in vivo,* situation. For a detailed overview be referred to Boots et al. (2008).

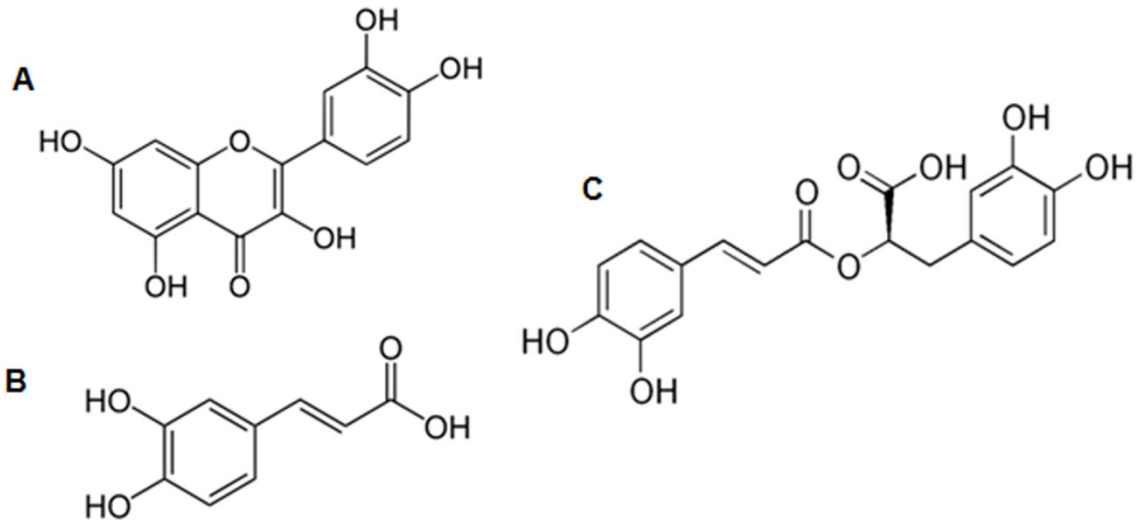



**Fig. 1 Chemical structure of (A)** Quercetin (MW: 302.236 g/mol), **(B)** Caffeic acid (MW: 180.16 g/mol), **(C)** Rosmarinic acid (MW: 360.31 g/mol)

**The phenolic acids: Caffeic acid and Rosmarinic acid**

Two classes of phenolic acids can be distinguished: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxycinnamic acids are more ubiquitous than the hydroxybenzoic acids and consist mainly of *p*-coumaric-, caffeic-, ferulic-, and sinapic- acids. They are found in almost every plant (Herrmann, 1976; Kuhnau, 1976).

**Caffeic acid** (CA, Fig. 1 B), in its free as well as esterified form, is the major representative phenolic acid and depicts between 75% and 100% of the total hydroxycinnamic acid content of most fruits (Manach et al., 2004). It occurs in edibles chiefly as an ester with quinic acid, called chlorogenic acid. Coffee is a major source of chlorogenic acid in the human diet; other sources include apples, pears, berries, artichoke and aubergines (Clifford, 1999). The absorbance of CA in humans could be shown by Olthof et al. (2001). It presents antioxidant properties *in vitro* (Castelluccio et al., 1995; Rice-Evans et al., 1996), as well as inhibitory activities against Gram-positive and -negative microorganisms (Lee and Lee, 2010). Furthermore it might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds (Kono et al., 1995). CA exhibits antiproliferative effects in various cancer cellines (for review see Jaganathan and Mandal, 2009), antiviral activities (for review see Arakawa et al., 2009), as well as antifungal drug potential (Pukkila-Worley et al., 2009). Moreover CA and derivatives prevent acute and chronic liver injury by inhibition of NF-kappaB (Muriel, 2009); it inhibits DNA methylation (a common characteristic in tumor cells) in cultured MCF-7 and MDA-MB-231 human cancer cells (Vucic et al., 2009). The *in vivo* impact on whole animal models is rarely investigated.

**Rosmarinic acid** (RA, α-*O*-caffeoyl-3,4-dihydroxyphenyl lactic acid; Fig. 1 C) is a polyphenol antioxidant carboxylic acid found in many *Lamiaceae* herbs, used commonly as culinary herbs such as lemon balm, rosemary, oregano, sage, thyme and peppermint. RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Scarpati and Oriente, 1958), and therefore belongs to the cinnamate conjugates (Clifford, 1999). A multitude of biological activities have been described, such as anti-depressive (Takeda et al., 2002), hepatoprotective (Osakabe et al., 2002), anti-inflammatory (Osakabe et al., 2004), anti-angiogenic (Huang and Zheng, 2006), antitumor (McKay and Blumberg, 2006), HIV-1-inhibiting (Dubois et al., 2008) and anti-mutagenic properties (Vattem et al., 2006; Furtado et al., 2008). RA is also known to possess marked antioxidant properties as a reactive species scavenger and lipid peroxidation inhibitor

(Kosar et al., 2008). The anti-inflammatory impact is thought to be at least partially based on the interference of RA with the complement cascade (Rampart et al., 1986; Englberger et al., 1988; Peake et al., 1991). Baba et al. (2004) found in a study with RA orally administered rats that the majority of fed RA was absorbed and metabolized as conjugated and/or methylated forms. So far most studies focus only on single outputs, but whole animal studies are missing.

## 1.3 The model organism Caenorhabditis elegans

**Fig. 2 *Caenorhabditis elegans*,** hermaphrodite on the 7$^{th}$ day of adulthood.

*History:* Sydney Brenner introduced *Caenorhabditis elegans* (*C. elegans*; *Caeno*, recent; *rhabditis*, rod; *elegans*, nice; Fig. 2) 1963 as a convenient model organism for development and neurology in biological research (Brenner, 1974). Since then *C. elegans* has been used as a research tool around the world (Wood, 1988) and a broad knowledge infrastructure has developed (e.g. WormBase, a database of *C. elegans* genetic and genomic information, curated by a consortium of researchers; wormbook.org, and others). Discoveries made with *C. elegans* have been awarded with Nobel Prizes in 2002 (genetics of organ development and programmed cell death), in 2006 (RNA interference) and in 2008 (work on GFP, some of it in *C. elegans*).

*Biology: C. elegans* is an originally free living, soil-dwelling, bacteriovorous (Byerly et al., 1976), non-parasitic, small (adult size about 1 mm) nematode. It has a short life span of approximately 18–20 days depending on the temperature (due to its ectothermie, Klass, 1977) and a rapid generation time (3 days at 22°C), thus, can be easily and cheaply cultured and preserved (cryogenically at -80°C) in large numbers in the laboratory. Adult wild type *C. elegans* hermaphrodites constantly contain 959 cells (cell constance), exactly 302 of which are neurons. The situation in males is somewhat different (Sulston and Horvitz, 1977; Sulston et al., 1980). Due to its transparency in all life-stages, it is easy to track cells and follow cell lineages, as the complete cell-lineage is well-established (Sulston and Horvitz, 1977). Hence, research on development and morphology of a single cell and how genes influence cell fate, can be done within a living organism (Kenyon, 1988; Wood, 1988; Donald, 1997; Horvitz, 2004). Furthermore, many green-

fluorescence protein (GFP) reporter-gene strains have been developed, enabling researchers to visualize signaling pathways, developmental processes and structures of anatomy (Gruber et al., 2009).

*Genetics:* As the first animal, *C. elegans'* whole genome was sequenced at the end of 1998 and striking similarities to human genes have been found (around 60-80%; Sonnhammer and Durbin, 1997; The *C. elegans* Sequencing consortium, 1998; Lai et al., 2000; Kuwabara and O'Neil, 2001; Harris et al., 2004), what made *C. elegans* a popular model for human diseases (e.g. diabetes and obesity, aging, cancer, Alzheimer's disease, Parkinson's disease, Huntington's disease, depression, neuronal regeneration/pain, autosomal dominant polycystic kidney disease (ADPKD), muscular dystrophy, ionchannelopathie and innate immunity (for excellent review see Kaletta and Hengartner, 2006)). Even though a direct translation of human pathology into *C. elegans* phenotypes is not possible, this model system approach helps to enlighten molecular mechanisms which contribute to find new therapeutics.

The size of the genome is around a hundred million base pairs (20x bigger than in *E. coli*, 1/30 of humans; www.wormclassroom.org), which codes approximately 18 000 genes. It has five pairs of autosomes and one pair of sex chromosomes. The distinction in two sexes (males and hermaphrodites) results from the ratio of sex chromosomes to autosomes ($6^{th}$ chromosome pair XX: hermaphrodite; X0: male). On one hand hermaphrodites can self-fertilize or mate with males, but hermaphrodites cannot fertilize each other. In case of mating with males, the ratio between males and hermaphrodites will be 50:50. In nature and under laboratory standard conditions, hermaphrodites are the most common sex (> 99%). To produce certain desired genotypes for genetic studies in the laboratory, reproduction can be channeled either to self-fertilization or crossing with males. By self-fertilization the diversity of individual genotypes can be neglected, thus populations can be maintained isogenic (clonal), which bears many advantages. A self-fertilizing hermaphrodite can produce up to 350 progeny during the first week of adulthood, a number which is even higher when mated with males. All of these characteristics contribute to the ease in producing various geno- and phenotypes for research issues (Klass, 1977; Wood, 1988; Horvitz, 1997). Worms can additionally be manipulated by the use of RNA interference (RNAi). Altered bacteria, producing dsRNA from a desired *C. elegans* gene, can be simply fed to the worms, what produce loss-of-function phenotypes in the nematode (Timmons and Fire, 1998).

*Advantages of research in* C. elegans*:* Due to the contribution of the whole *C. elegans* research community an extensive library of mutant- and GFP strains, as well as RNAi bacterial strains have been generated, which can be ordered (*C. elegans* Genetic Center and e.g. under www.lifesciences.sourcebioscience.com). Furthermore, many protocols and methods for genetic manipulations have been developed and are easy to access through databases. Hence, research in *C. elegans* bears the potential to bridge the gap between *in vitro* and *in vivo* approaches by combining the use of high-throughput technologies (e.g. genome-wide RNAi studies, microarrays) in a multicellular, whole animal setting (Kaletta and Hengartner, 2006) and therefore is also a feasible model for the pharmaceutical industry to identify new drug targets.

### 1.3.1 *C. elegans* in aging research

*C. elegans* undoubtedly gained its popularity in aging research due to the findings of single gene mutations, which dramatically prolonged the lifespan of the nematodes. By then, the aging research area was predominantly circulating around the underlying genetics, while now many more approaches are used, e.g. environmental manipulations such as caloric restriction (CR) and hormetic treatments, evolutionary and population studies, models of age-related diseases, and drug screening for compounds that extend life span (reviewed in Olsen et al., 2006).

*Qualifications for being an aging model:* Age-related changes in *C. elegans* resemble those in other organisms: nematodes are less active, display uncoordinated movements and eventually completely stop moving, likely due to muscle degeneration. Furthermore, they accumulate lipofuscin, dark-pigments and vacuole-like structures (Herndon et al., 2002) and display enhanced levels of oxidized proteins (Adachi et al., 1998; Nakamura et al., 1999; Yasuda et al., 1999). Although *C. elegans* populations are comparatively clonal, the variability of age-related changes between individuals are still very high, which leads to the assumption that stochastic events play a prominent role in *C. elegans'* aging (Herndon et al., 2002).

Similar to several other species, in *C. elegans* there is a mechanistic connection between stress resistance and longevity that is well established. It is probable that all long-lived mutant strains are armed with a robust resistance to oxidative stress, hypoxia, heat shock, UV radiation and heavy metal stress. Accordingly, it is possible to enhance longevity in wild type animals by a range of manipulations that enhance stress resistance, e.g. overexpression of certain heat shock proteins (*hsps*). Furthermore, longevity in wild type animals can be triggered by the short-term application of stressors such as increased heat, high oxygen and gamma irradiaton, (reviewed in Olsen et al., 2006). These aspects all contribute to the feasibility of *C. elegans* as a model organism for aging research.

*Genetic pathways affecting aging in C. elegans:* The existence of stress-resistant, long-living dauer larvae formed as a result of stressful conditions such as low nutrition or high population density, is of great interest for aging research (Anderson, 1978; Larsen, 1993; Riddle and Albert, 1997). It has been found that many mutations with effects on dauer formation are also players of the evolutionary conserved insulin/IGF-I signaling pathway (Insulin-like signaling; ILS; Fig. 3) and affect adult longevity (reviewed in Kenyon, 2011). Many ILS pathway mutations have been shown to influence lifespan in flies and mice and recently new links to human longevity have been reported (Kenyon, 2011). Key genes in ILS are *daf-2*, *age-1* and *daf-16*. *daf-2* encodes a homolog of the mammalian insulin/IGF-I receptor (Brown, 1979) and *age-1* encodes the catalytic p110 subunit of phosphoinositide-3-OH kinase (PI3K) found downstream of DAF-2 (Garigan et al., 2002). Mutations in *age-1* and *daf-2* cause large increases in the nematodes' lifespan as well as enhanced stress resistance, both dependent on *daf-16*. *daf-16* encodes the central FOXO forkhead transcription factor of the ILS pathway and is the major downstream effector of DAF-2 (Tatar et al., 2003). Its transcriptional targets include stress response, antimicrobial, and metabolic genes, as well as many genes of unknown function (for detailed review see Landis and Murphy, 2010), some of which in turn impact aging (Murphy et al., 2003). Genes which also play a role in ILS are *sgk-1*, *aap-1, daf-18, pdk-1*, *akt-1/2*, *hsf-1* (reviewed in Schaffitzel and Hertweck, 2006).

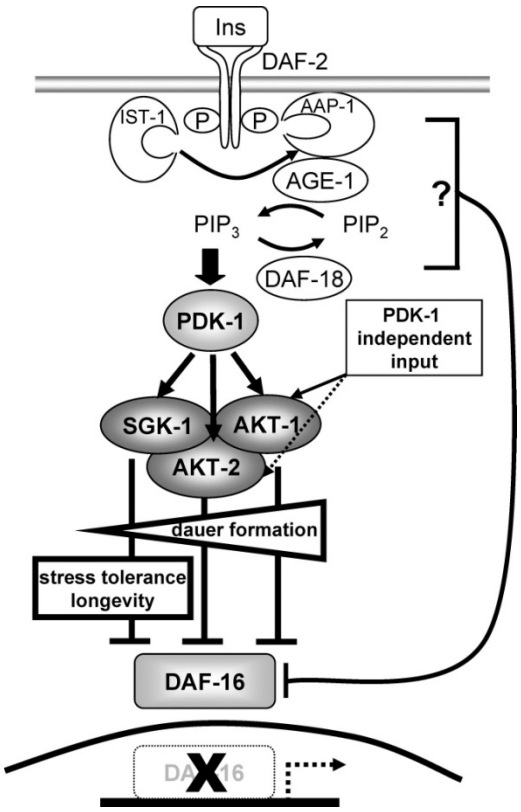



**Fig. 3 Model for ILS Signaling.** Upon DAF-2 activation, AGE-1 and PIP3 activate PDK-1. PDK-1 phosphorylates AKT-1, AKT-2, and SGK-1 that form a kinase complex to antagonize DAF-16 by direct phosphorylation. The major importance of AKT-1 and AKT-2 to prevent DAF-16 from inducing the dauer program is indicated. SGK-1, but not the AKT kinases, inhibits DAF-16-induced longevity and stress tolerance. (Reprinted from Hertweck et al. (2004), with permission from Elsevir).

Beside the ILS and its molecular players, more genes and genetic signaling pathways have been discovered related to lifespan variations (Fig 4). To mention a few: germline signaling (*daf-12 glp-1*, *mes-1*), CR, SIR2 deacetylase activity (probably with importance in CR), JNK-signaling (*jnk-1*, *jkk-1*) , TOR (*let-363*, *daf-15*, *pep-2*) and TUBBY signaling (*tub-1*), mitochondrial mechanisms (*isp-1*, *clk-1*), oxidative stress signaling (*gsk-3*, *skn-1*) and others (*aak-2*, *eat-2*). For detailed review be referred to Schaffitzel and Hertweck (2006).

More recently, the identification and analysis of compounds that delay aging and extend lifespan became an important aspect of gerontology research. Besides testing theories of aging, it leads to the identification of endogenous mechanisms which underlie the aging process and provides a basis for treatments, which are able to slow down senescence. A good overview about the procedure of testing and a good review about so far conducted studies are provided in Collins et al. (2006).

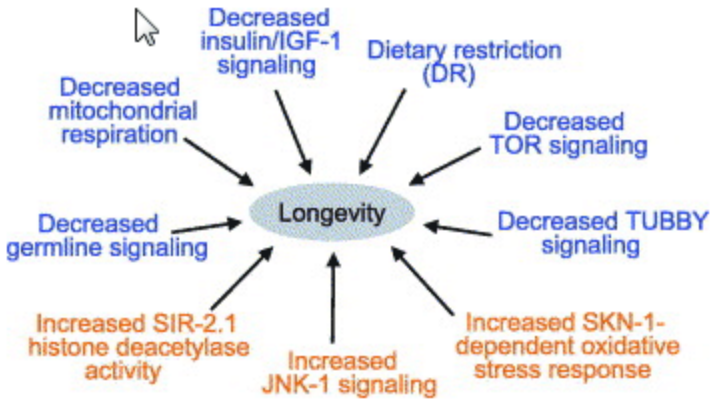



**Fig. 4 Regulation of lifespan in *C. elegans*.** Several evolutionary conserved mechanisms contribute to the complex regulation of lifespan in *C. elegans*. Decreased activity (indicated in blue) and increased activity (indicated in orange) of the following signaling pathways and processes lead to longevity in the animal. In some of these mechanisms, including ILS-, germline-, TOR-, TUBBY-, JNK- signaling, and SIR2 activity, lifespan extension occurs in a DAF-16-dependent manner. (Reprinted from Schaffitzel and Hertweck (2006), with permission from Elsevir).

## 1.4 Aging

*"The situation borders on ludicrous. We have landed on the moon and sequenced the human genome but there is still no scientific agreement on even the nature of aging."* (Goldsmith, 2008)

Aging, also called organismal senescence, is due to the accumulation of changes in an organism over time (Bowen and Atwood, 2004). These changes affront the healthy functioning and survival of the organism; however, it has proved difficult to deduce the relative importance of the manifold processes that leads to aging (Murphy and Patridge, 2008). Commonly aging is characterized by a declining ability in stress response, decreased homeostatic balance and an increasing risk of aging-associated diseases, which finally leads to death. Species-specific maximum lifespan correspond to inherited differences in the rates of aging and therefore a human is considered elderly at 80 years, while a mouse is considered elderly at 3 years of age. From a more generalized view, organismal aging could be summarized as the result of two contrarian forces: (i) destabilization processes that increase the probability of death of an organism, and (ii) longevity assurance mechanisms that prevent, repair, or embank damage (Zimniak, 2008). Processes of the first group are often chemical and physico-chemical in nature and are either inevitable or only under marginal biological control. Unlike protective mechanisms, which are genetically determined and are subject to natural selection. Lifespan is therefore largely dependent on the investment into protective mechanisms, which evolved to optimize reproductive fitness. Senescence of the organism gives rise to the Gompertz-Makeham law of mortality, which postulates that mortality rate increases exponentially with age. It is composed of an age-independent component (Mekeham term, Makeham, 1860) and an age-dependent component (the Gompertz term, Gompertz, 1825). Where the age-independent component is negligible (e.g. laboratory conditions, low mortality countries), the function simplifies to the Gompertz law of mortality.

### 1.4.1 Why do we age? - Selected Aging theories

Why aging occurs has been debated for 150 years and still remains a mystery in the scientific world, with yet no consensus over the general nature of aging. Two general directions of thoughts exist, which can be summarized by following questions:

1. Is aging an evolved characteristic of organism's design, programmed by the organisms' genetics and thus the active result of this pro-aging design, because a limited life-span confers some advantage?
2. Or is aging not an evolved characteristic but rather a fundamental limitation that apply to all living things, a defect that confers no benefit and displays rather the passive result of forces acting upon the organism?

Around 300 different scientific aging theories have arisen so far (Medvedev, 1990) but all of them either fail to fully explain observed animal characteristics or conflict with the widely accepted evolution theory. Some of these theories may be a result of other theories and some are interlinked.

The existing aging theories can be crudely classified in three categories (http://www.programmed-aging.org/):

- **Mechanistic theories**
  - ***1.*** Simple deterioration theories ("wear and tear")
- **Evolutionistic theories**
  - ***2.*** Programmed theories, also known as adaptive or active theories
  - ***3.*** Non-programmed theories, also known as non-adaptive or passive theories

Below follows a selection and short overview of single theories.

**Simple deterioration theories ("wear and tear")**

Biological aging could be simply the result of universal deteriorative processes, such as oxidation or wear and tear, as is the cause in machineries and other inanimate objects. Some theories assume that aging is just a process of increasing entropy and over a certain time results in deterioration. However, entropy can be reversed by application of energy and living organisms routinely reverse entropy using food energy in order to grow and live. But why is the body able to use energy to antagonize this deterioration for a certain time, but fails in old age? And how is it possible, that two closely related species, like naked mole rats and mice for example, display huge variations in their maximal lifespan (30 and 2 years, respectively)? Furthermore these theories fail to explain, why some organisms apparently do not age (Negligible Senescence, e.g. Hydra; Martinez, 1998) and why other organisms die suddenly after reproduction. Consequently, few scientists believe in simple deterioration theories and more complex explanations for aging evolved. Nevertheless, many scientists do still believe in oxidative and other molecular damage to be involved in the aging process, but beyond additional factors probably determine whether this damage is repaired.

*ROS and aging:* Oxygen is essentially required for aerobic life, but also may play a prominent role in aging (for a detailed review of causes and effects of ROS and a summary of experiments in the worm *C. elegans* that have examined the relationship between ROS and aging see Van Raamsdonk and Hekimi, 2010). Following the aging theories associated with **Reactive oxygen species** (ROS) are scarcely described:

In the early 1900s observations by Rubner pointed towards a role for oxygen in determining lifespan. He found an inverse relationship between metabolism and lifespan: large animals tend to have longer lives, than small animals and small animals have a higher metabolic rate (Rubner, 1908). Hence, the basis for the **Rate-of-living theory of aging** was born, which postulates that the lifespan is dependent on the metabolic rate (Pearl, 1928). Furthermore this theory tried to include the idea of a delimited amount of energy which each individual own when starting life. The lifespan is therefore determined by the speed of energy consumption (for review of the rate-of-living theory see Speakman, 2005).

The **Free radical theory of aging (FRTA),** proposed by Harman (1956), provided a possible mechanism for the rate-of-living hypothesis: it suggested that the progressive process of aging results from the accumulation of molecular damage caused by ROS, by-products of normal metabolism. During energy generation from food and oxygen, a small percentage of oxygen is converted to ROS. ROS causes oxidative damage to DNA, proteins, and lipids, which accumulates over time. The free radical theory of aging suggests that the accumulation of oxidative damage is the cause of aging. According to this theory, the accumulated oxidative damage eventually leads to cellular dysfunction and increases the probability of death. Hence, a species with a higher metabolic rate, produce more ROS, thereby more molecular damage arise faster, the rate of aging increase, thus the lifespan decreases (for review see Van Raamsdonk and Hekimi, 2010).

In 1972 Harman refined his theory to highlight the role of mitochondria in the aging process (**Mitochondrial theory of aging (MTA),** also called Oxidative stress theory of aging). The energy providing mitochondria are the main producers of free radicals and also the primary sites for free radical damage. The production of free radicals at the level of the electron transport chain subsequently results in the formation of various ROS species in the mitochondria of all tissues (Lapointe and Hekimi, 2010) and damage in mitochondrial and cytosolic constituents occur which lead over time to aging. Furthermore it is suggested that the oxidative damage to mitochondrial constituents results in a deterioration of mitochondrial function. The resulting mitochondrial dysfunction leads to more ROS generation and the global (also non-mitochondrial) oxidative damage will progressively accumulate.

In the last decades, both the FRTA and the MTA has attracted much interest and hence are presumably the broadest tested theories of aging. Numerous experiments were conducted, both supporting and refuting the assumptions of these theories (reviewed in Muller et al., 2007; Lapointe and Hekimi, 2010). Nevertheless, there is unquestionable evidence for the age-dependent accumulation of ROS damaged products (Epstein et al., 1972; Adachi et al., 1998; Gerstbrein et al., 2005;), abnormalities and dysfunction of mitochondria as well as a decreased metabolic capacity (Epstein et al., 1972; Melov et al., 1995; Van Voorhies and Ward, 1999; Yasuda et al., 2006; Braeckman et al., 2002) in *C. elegans* (reviewed in Gruber et al., 2009). Reasons to refute these theories arose due to discoveries of ROS not just as damaging species, but also as important signaling molecules in intracellular pathways and as effectors in pathogen resistance (Lamb and Dixon, 1997). Hence, a consensus on whether or not oxidative damage is responsible for aging has not yet been achieved.

The FRTA states that ROS are the major cause of aging. If this is true, the modulations of endogenous antioxidant defense systems or a supplementation with exogenous antioxidants seem to be promising strategies for delaying aging (Gruber et al., 2009). Indeed, a number of studies have examined the effects of compounds that influence ROS levels on *C. elegans* lifespan (re-

viewed in Collins et al., 2006 and Ramsdoonk and Hekimi, 2010) and also mutations which result in increased or decreased antioxidant defense (reviewed in Ramsdoonk and Hekimi, 2010).

*Mutations affecting the endogenous defense: C. elegans* mutants which support the FRTA are e.g. *mev-1*, *daf-2*, *eat-2* and *age-1*. The *mev-1* mutation affects the mitochondrial complex II, ROS production is increased and levels of oxidative damage are elevated. *mev-1* are hypersensitive to oxidative stress and short-lived (Senoo-Matsuda et al., 2001). On the other hand long-lived *daf-2*, *age-1* and *eat-2* display elevated levels of antioxidant enzymes like catalases (CAT) and superoxide dismutases (SOD) and possess an enhanced resistance to oxidative stress (Larsen, 1993; Vanfleteren, 1993; Houthoofd et al., 2002; Murphy et al., 2003; McElwee et al., 2004; Panowski et al., 2007). Further examples of mutants with an inverse relationship of lifespan and sensivity to oxidative stress are *prdx-2*, *trx-1* and *gst-10* (reviewed in Van Ramsdoonk and Hekimi, 2010). Hints against the FRTA comes from experiments with *sod* deletion mutants: the deletion of *sod* genes, alone or in combination, has little or no impact on lifespan despite the clear increase in sensitivity to oxidative stress (reviewed in Gruber et al., 2009 and Van Ramsdoonk and Hekimi, 2010). All results from mutant strains have to be interpreted carefully, since some of these antioxidant defense genes have multiple roles within the cell (e.g. peroxiredoxin, TRX, glutaredoxin) whereas others have the only (known) function to detoxifying ROS (e.g. SOD and catalase). Thus, the effect on lifespan may not necessarily result from the gene's effect on oxidative stress resistance.

*Studies with antioxidative acting compounds (exogenous antioxidative defense):* Many studies examined the effects of so-called antioxidants (or compounds which influence ROS levels) on *C. elegans* lifespan (summarized in Collins et al., 2006; Saul et al., 2009; Van Ramsdook and Hekimi, 2010). While treatment of *C. elegans* with exogenous antioxidants predominantly provoked an increased resistance to oxidative stress, there was some variability concerning the effects on lifespan (dependent on dose, experimental conditions, and method of delivery). Nevertheless, most of the tested antioxidants could increase lifespan under at least one set of conditions. Examples for such compounds are EGCG (Brown et al., 2006; Abbas and Wink, 2009; Zhang et al., 2009); Resveratrol (Gruber et al., 2007); Gingko Bilboa (Wu et al., 2002; Cao et al., 2007), Tocotrienol (Adachi and Ishii, 2000), coenzyme Q 10 (Ishii et al., 2004) and others.

Recently some artifacts associated with the handling of *C. elegans* in testing of antioxidants and other agents have been discussed (Gruber et al. 2009; Pun et al. 2010). First, *in vitro* determination of antioxidants is not predictive to *in vivo* antioxidant properties. Second, *in vivo* antioxidant properties as measured e.g. by protein carbonyl content were not predictive for lifespan extension (Pun et al., 2010). And third, Pun et al. suggests, the ability of (some *in vitro*) antioxidants to increase lifespan is not solely explainable by their mediation of oxidative stress resistance. Other mechanisms have to be considered to explain these findings. For example issues about the direct *vs.* the indirect antioxidative (triggering the endogenous antioxidative response; elevated expression of enzymes) actions of the compound *in vivo*. One argument for indirect actions recently was pointed out by Halliwell (2007): unconjugated polyphenols, which possess antioxidant properties, rarely exceed the low µM range in plasma, while total antioxidant capacity is often in the range of 1 mM. These pitfalls and the associated complexities can only be circumvented by choosing a broad spectrum of bioassays and adequate biomarkers for oxidative damage and protection. Furthermore methodological challenges have to be addressed which ensure the standardization of handling in the *C. elegans* community to circumvent variations of results due to variable test designs (information on methodological considerations, genetic inhomogeneity and drift in *C. elegans* can be found in Collins et al. (2006), Patridge and Gems (2007) and Gruber et al., (2009)).

Finally it should be mentioned, that extensive studies addressing the supplementation of antioxidants in humans have failed to support assumptions made by FRTA (Anghel, 2010). The current scientific consensus is rather that there is no health benefit when taking antioxidant supplements (Howes, 2006) or even more unexpected an article announced that antioxidants may actually prevent the health-promoting effects of physical exercise (Ristow et al., 2009). Howes (2006), who reviewed clinical studies with altogether 550 000 humans, which overwhelmingly have failed to confirm the FRTA, claims: “The free radical theory has fallen”. Probably studies with humans bear too many variables to get predictive results. For a critical review see Halliwell (2007). A new concept arose: the mitohormesis theory.

Contrasting to assumptions made by the FRTA, **mitohormesis (mitochondrial hormesis)** denotes a biochemical process, in which an enhanced production of ROS in activated mitochondria, induce the cell intern stress response against oxidative stress, thereby reduce net stress levels and hence, possibly prolongs lifespan in a hormetic way (Vanfleteren, 1993; Houthoofd et al., 2002; Johnson et al., 2002; Kharade et al., 2005; Sinclair, 2005; Zarse et al., 2007). Further support gains this theory by evidence of increased ROS production provoked by physical exercise (Chevion et al., 2003), which has proved to prolong lifespan in humans (Lindsted et al., 1991; Manini et al., 2006). Schulz et al. (2007) could show that increased glucose availability decreases *C. elegans* lifespan, while controversially, diminished glucose metabolism extends life by inducing mitochondrial ROS production and thereby stress resistance. Recently, a study by Ristow et al. (2009) in humans evaluated the influence of the antioxidants Vitamin C and E on the positive effect of physical exercise. They conclude that antioxidant supplements prevent the induction of molecular regulators of insulin sensitivity and endogenous antioxidant defense by physical exercise. Consistent with the concept of mitohormesis, they postulate that temporary enhanced oxidative stress levels are presumably a potentially health promoting event at least concerning insulin sensitivity and the prevention of insulin resistance and type 2 diabetes mellitus. Additionally, the meta-analysis of studies in humans with antioxidant supplements (Howes, 2006; Bjelakovic et al., 2007), could not confirm benefits, but rather neglects them. In summary, all these findings support the mitohormesis theory.

Gems and McElwee (2005) postulated the **Green theory of aging** (Fig. 5) on the basis of findings in DNA-microarray studies in long-lived *daf-2* mutants (McElwee et al., 2003, 2004; Murphy et al., 2003). They found a major role in longevity assurance of the phase 1 and phase 2 detoxification system in *daf-2* mutants, which involve cytochrome P450 (CYP), short-chain dehydrogenase/reductase (SDR) and UDP glucuronosyltransferase (UGT) enzymes. This system is energetically costly and requires the excretion of its products out of the cell (unlike superoxide and hydrogen peroxide detoxification). Given that aging results from molecular damage by toxic products of the metabolism and xenobiotics (whereof ROS are just one component), the enhanced activity of detoxification processes can lead to an extended lifespan. A further component of this theory is based on the conservation of existing proteins via molecular chaperones. Due to the accentuation in this theory on investment in cellular waste disposal and on protein conservation, Gems and McElwee (2005) named this longevity assurance mechanism the green theory.

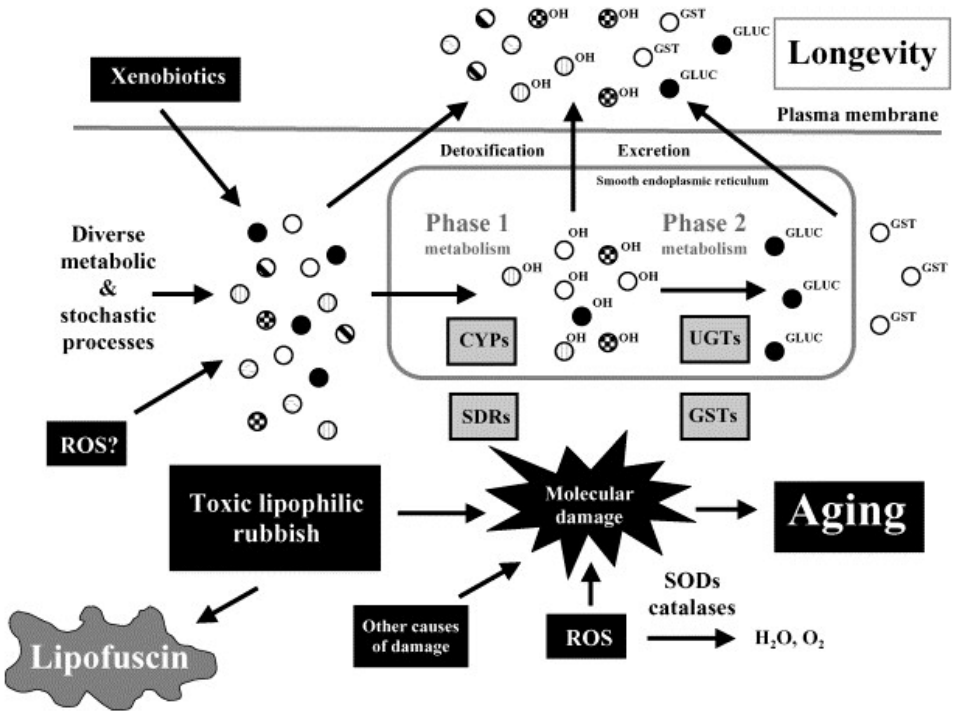



**Fig. 5 Broad spectrum detoxification and longevity assurance.** It is proposed that a major contributor to the aging process is molecular damage resulting from toxins (mainly endobiotic and lipophilic) which are targets of the phase 1, phase 2 detoxification system. In this model, the smooth endoplasmic reticulum functions as a longevity organelle, derivatising and excreting lipophilic toxins. However, this process is energetically costly, and most cells take the energy saving step of down-regulating the detoxification system, and dumping toxins within lipofuscin depots. The consequence of this is accelerated aging. Reversing this process means up-regulation of the biotransformation and hsp-response (Green theory of aging). CYP: cytochrome P450; GST: glutathionyl; Gluc: glucuronosyl/glucosyl; SDR:short-chain dehydrogenase/reductase; UGT:UDP-glucuronosyl-/glucosyl- transferases. (Reprinted from Gems and McElwee (2005), with permission from Elsevier).

## Programmed, adaptive, active theories

These theories assume that organisms are designed with a genetically programmed limited lifespan because aging and consequently limited lifespan conduce to some evolutionary advantage. According to this concept, aging is a necessary biological function and like other biological functions, can be directed by a lifespan regulation system involving hormones, signaling, genes, sensing of external conditions. A lifespan exceeding the species-specific value mediate disadvantages and therefore cause evolutionary motivation to develop the aging function.

Weismann (1882) suggested that aging and finally death was a genetically programmed, evolved characteristic due to natural selection, because it confers benefit to the species in spite of its negative effects on the fitness of the individual (**Weismann's Theory of Programmed Death**). By removing older members, programmed death shifts resources (e.g. food and habitat) from less evolved, older animals to more evolved, younger animals and thereby improves the species' general ability to evolve, especially its ability to adapt to changes in its external environment. Because of two problems, current biologists have largely discounted Weismann's theory: (i) If there was a genetically programmed characteristic, its mutation would possibly lead to immortal individuals, and therefore evolution would tend to infinite longevity, which obviously has not happened so far. (ii) Most animals in the wild essentially never lived long enough to die of old age. Therefore the potential benefit of programmed death could never have been realized

and thus could not have driven evolution. Programmed death therefore violates the theory of natural selection.

**Non-programmed, non-adaptive, stochastic passive theories**

Main focus of these theories is embedded in the assumption that aging does not serve an evolutionary goal. Aging is rather an inevitable adverse side-effect of some useful biological functions. These theories assume that organisms do not have an evolutionary need to live longer than the species-specific lifespan and therefore did not develop or even lost the trait to live longer.

Medawar (1952) postulated that the evolutionary effect of adverse conditions declines to the point of being negligible, beyond the age at which an organism is initially capable of reproduction. Therefore, random, detrimental mutations which only cause late-life negative effects would not be ruled out efficiently by natural selection and can accumulate over time and with each generation (**Mutation accumulation theory**). Hence they would cause the decline and damage that we associate with aging (Edney and Gill, 1968). Among other aspects, this theory cannot explain, why a number of diverse organisms (e.g. salmon, octopus, marsupial mouse and bamboo) also display death closely following an act of sexual reproduction. Death in these species appears to be controlled by the reproductive function or controlled by factors triggering reproduction as opposed to calendar age.

Pleiotropy is defined as a situation in which a single gene controls more than one trait. Williams (1957) proposed that aging was caused by the combined effect of many pleiotropic genes that each displayed beneficial effects in an animal's youth but also had an adverse side effect at older age (**Antagonistic pleiotropy theory**). If evolution is a race to have the most offspring the fastest, then enhanced early fertility could be selected even if it is associated with trade-offs that included deterioration, senescence and death later on. The antagonistic pleiotropy theory considered any alteration of the aging process as impossible, because aging is held for a side effect of necessary functions. One example on which this theory is applicable is p53, which suppresses cancer during lifetime, but also suppresses stem cells, which recreate worn-out tissue and thereby causing aging (Rodier et al, 2007). Contrary examples brought breeding experiments with Drosophila. Leroi et al. (1994) inbred flies which lived twice as long as their parental generation, but nevertheless laid more eggs, thereby invalidating antagonistic pleiotropy.

Proposed by Kirkwood and Holliday (1979), the **Disposable soma theory** assumes that aging occurs due to the accumulation of damage over time, which results in failures of repair and defense mechanisms (Kirkwood, 1977; Kirkwood and Holliday, 1979; Kirkwood, 2000). They presumed that certain gene mutations save energy for reproduction in an antagonistic pleiotropic manner, by partially disabling molecular proofreading and other maintenance mechanisms in somatic cells (reviewed in Ljubuncic and Reznick, 2009). That means an organism has to deal with delimited resources, which have to be rationed between maintenance and reproduction. Thus, an optimal balance between somatic repair and maintenance on one hand versus reproductive success on the other hand is to be aspired by each organism. The germ line (reproductive cells) must be maintained to preserve the viability throughout many generations, whereas the soma serves only one single generation. Therefore, the disposable soma theory links mechanistic and evolutionary theories of aging by stating that aging results from progressive accumulation of molecular and cellular damage, as a direct consequence of evolved limitations in the genetic settings of maintenance and repair functions. Supporting evidence for this theory

comes from studies in mice and *Drosophila melanogaster*, where a reduction in body size correlates with longevity (Flurkey et al., 2001; Nielsen et al., 2008) but also in *C. elegans*, where Harrington and Harley (1988) observed a reduction of offspring in response to Vitamin E mediated longevity. Similarly, Saul et al. (2009, 2010) discovered that Catechin and Tannic acid mediated longevity was associated with an attenuation of growth.

### 1.4.2 Modulation of lifespan: Caloric restriction and Hormesis

**Caloric restriction (CR)**

In 1935 McCay et al. found, that rats fed a restricted diet show diminished age-associated physiological deterioration and partly age-associated diseases, and display enhanced lifespan. Since that time CR (also called dietary restriction) has been grown to a major research area in gerontology and it has been shown that application of a 30-50% restricted diet is able to extend lifespan in multiple species and model organisms (rats, mice, fish, flies, nematodes, water fleas, yeast, dogs, spiders; Masoro, 2000). Some side-effects of CR have been reported e.g. limitations in the reproductive capacity (Holliday, 1989; Vanfleteren & Braeckman, 1999; Martin et al., 2008) and the growth (McCay et al., 1935; Mörck & Pilon, 2006). Until now many reviews focusing on CR have been published (to cite a few: Weindruch and Walford, 1988; Masoro, 2000; Speakman et al., 2002; Heilbronn and Ravussin, 2003; Ingram et al., 2004; Anson et al., 2005; Gredilla and Barja, 2005; Sinclair, 2005; Wolff and Dillin, 2006; Bishop and Guarente, 2007 and Piper and Bartke, 2008).

*Experimental induction of CR*: A number of methods exist to restrict nutrients, all of which provoke longevity (Goodrick et al., 1990; Mair et al., 2005; Masoro, 2005; Dilova et al., 2007; Mair and Dillin, 2008; Piper and Bartke, 2008; Skorupa et al., 2008). Considering *C. elegans* there are eight different protocols (genetic mutation in the *eat-2* gene, two different methods to dilute the bacteria in liquid cultures, axenic medium, chemically defined medium, dilution of peptone in the agarose plates, absence of bacteria on the plates and serially diluted bacteria on plates; Greer and Brunet, 2009). Moreover, a few chemical compounds have to be proposed to act as CR mimetics without actually restricting food (Ingram et al., 2006). In this context resveratrol was claimed to mimic CR in yeast (Howitz et al., 2003), worms (Wood et al., 2004; Viswanathan et al., 2005; Gruber et al., 2007), flies (Wood et al., 2004), fish (Valenzano et al., 2006) and mice (Baur et al., 2006), although some studies could not find any life extension in flies (Bass et al., 2007) and mice (Pearson et al., 2008). At present, studies with primates and humans are ongoing and will give final results in a few years. So far humans on a CR diet were found to display a reduction in blood pressure, glucose levels, insulin levels, body mass index, body fat percentage, C-reactive protein and other markers (Fontana et al., 2004). Thus, risk factors for age-related diseases like diabetes and arterioscleroses are lowered and it appears possible that CR extends life span also in these species (Fontana et al., 2004; Heilbronn et al., 2006; Ingram et al., 2006a; Weindruch, 2006 and Fontana and Klein, 2007).

*General mechanisms underlying CR-induced longevity*: Many hypotheses regarding the underlying mechanisms have been postulated (reviewed in Sinclair, 2005), which include developmental delays (McCay et al., 1935), reduced metabolic rate (reviewed in Krystal and Yu, 1994), laboratory artifacts (Cutler, 1982; Hayflick, 1994), attenuated glucocorticoid pathways (Spindler et al., 1991; Nelson et al., 1995; Masoro, 1996), diminished fat levels (Berg and Simms, 1960), decreased ROS (reviewed in Barja, 2004; Merry, 2002), increased rate of cell survival (Koubova and Guarente, 2003; Cohen et al., 2004), changes in protein turnover rates (Lewis et al., 1985; Tavernarakis and Driscoll, 2002; Del Roso et al., 2003), diminished glucose and insulin levels

(reviewed in Kalant et al., 1988), diverse endocrinological changes (subject of debate in Mobbs et al., 2001; Miller et al., 2002; Bluher et al., 2003; Masternak et al., 2004) and hormesis (Masoro and Austad, 1996; Rattan, 2004; Turturro et al., 2000; Masoro, 2000; Lithgow, 2001; Mattson et al., 2002; Anderson et al., 2003). The hormesis hypothesis (for general introduction to hormesis section below) of CR embrace many of aforementioned events by making four key predictions (Sinclair, 2005): (i) CR induce signaling pathways that respond to biological stress and a low nutritive status; (ii) these pathways contribute in defending against causes of aging; (iii) these pathways regulate glucose, fat, and protein metabolism to enhances the chance of survival during times of stress; and (iv) these pathways act in a coordinated manner due to endocrinological regulation.

*Genetic basics of CR:* Recent research uncovered genes which mediate longevity by different CR regimes or resveratrol in invertebrates (Lakowski and Hekimi, 1998; Wood et al., 2004; Hansen et al., 2005; Viswanathan et al., 2005; Wang & Tissenbaum, 2006; Bishop & Guarente, 2007; Greer et al., 2007; Hansen et al., 2007; Panowski et al., 2007; Steinkraus et al., 2008). Possibly the one that gained the most attention was the NAD-dependent deacetylase of the Sir2 family (Greer and Brunet, 2009). It was found to be necessary for longevity in various CR regimes and resveratrol (Lin et al., 2000; Rogina & Helfand, 2004; Wood et al., 2004; Chen et al., 2005; Viswanathan et al., 2005; Wang & Tissenbaum, 2006). Controversially, Kaeberlein et al. (2004, 2006), Lee et al. (2006), Bass et al. (2007) and Hansen et al. (2007) found no evidence for the involvement of Sir2 in CR or resveratrol-mediated longevity. Additionally the TOR pathway was identified in yeast, worms and flies as involved in CR action (Kapahi et al., 2004; Kaeberlein et al., 2005; Hansen et al., 2007), but apparently not in *C. elegans* (Henderson et al., 2006). More genetic mediators for CR are discussed in Greer and Brunet (2009).

## Hormesis

*"All things are poison, and nothing is without poison; only the dose permits something not to be poisonous."*
(Paracelsus, (1493 - 1541))

In 1888 hormesis was first described by Schulz, a pharmacologist who observed the stimulation of yeast growth after treatment with small concentrations of poisons. Together with Arndt, who studied the effect of a low drug exposure in animals, they formulated the Arndt-Schulz law. The rule was discredited in the 1920s and '30s due to Arndt's advocacy of homeopathy (Kaiser, 2003). 1943 the term hormesis was first coined by Southam and Ehrlich, but is recently revived due to the work of Calabrese on peppermint plants (Calabrese, 2004).

*Concept of hormesis:* Hormesis (from Greek hórmēsis "rapid motion, eagerness," from ancient Greek hormáein "to set in motion, impel, urge on") is a term that denotes adaptive non-linear, biphasic dose-response effects at low concentrations of otherwise toxic agents (Oberbaum and Cambar, 1994). It is characterized by low dose stimulation and high dose inhibition, typically resulting in either a J-shaped or an inverted U-shaped dose response (Fig. 6). It has been established in various fields of biology and proposed to have no taxonomic limitation. Any agent, be it physical, chemical or biological, may be expected to be stimulatory when it is given in much smaller doses than found to be harmful (irradiation, food limitation, heat stress, hypergravity, reactive oxygen species and other free radicals, chemical or natural compounds in food (hormetins) and others; Rattan, 2008). However, hormesis as a concept is controversially debated (Kaiser, 2003).

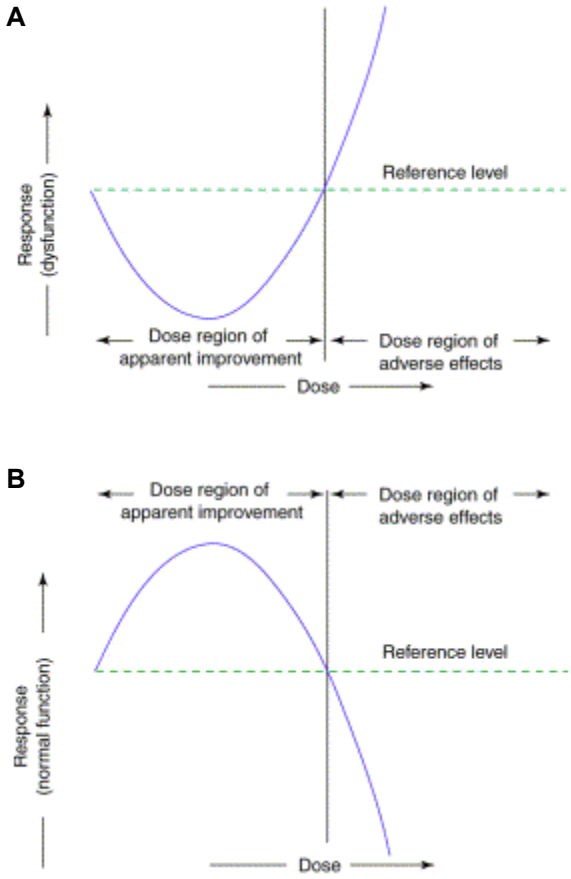



**Fig. 6 U-shaped dose–response (hormetic) curves** illustrating apparent **(A)** reduced dysfunction and **(B)** enhanced function. (Reprinted from Calabrese and Baldwin (2001a), with permission from Elsevir).

*Mechanisms of hormesis:* The beneficial effects of mild stress on aging and longevity have been studied for several years (Le Bourg, 2009). Mild stress appears to slightly increase longevity, delay aging, and increase resistance to adjacent severe stresses. It is suggested that these stimulatory effects can be generally explained by (i) a direct response to the stimulus or by (ii) an overcompensation response after exposure to a toxic agent which disturbs homeostasis (Calabrese and Baldwin, 1998; Calabrese, 2001). Detailed molecular actions of hormesis are becoming increasingly understood, and comprise a cascade of stress response and other pathways of maintenance and repair (Rattan, 2008). It is suggested, that during the long path of evolution organisms have developed complex mechanisms to cope with environmental hazards in order to protect themselves from becoming extinct: the hormetic response pathways (Mattson, 2008), which involve proteins such as ion channels, kinases, deacetylases, transcription factors for cytoprotective proteins, chaperones, antioxidant enzymes and growth factors (Mathers et al., 2004; Mattson et al., 2004; Young et al., 2004). Rattan (2008) suggests that the "chain of events following initial hormesis leads to biologically amplified effects, synergistic and pleiotropic." This phenomenon is termed "the homeodynamic space" (by means of an increased defense capacity and reduced load of damaged macromolecules) that is enhanced and prevents disease and diminishes the rate of aging. For a review of previous results concerning hormesis in various fields, e.g. radiation hormesis, caloric restriction hormesis, nutritional hormesis and hormetins, hypergravity hormesis, thermal and exercise hormesis, refer to Rattan (2008).

*Hormesis and risk-assessment:* In a literature search Calabrese and Baldwin uncovered few studies demonstrating hormesis (Calabrese and Baldwin, 2001b), e.g. plants exposed to herbicides or metals grow amply, bacteria prosper better in the presence of tiny doses of antibiotics, and arsenic treatment cause immune cells to proliferate faster (summarized in Kaiser, 2003). Some proponents of the concept of hormesis animate it should be attended as the norm, not the exception and claim that the risk-assessment guidelines should be reassessed. They propose it applies to all toxicants in the absence to the contrary (Axelrod et al., 2004). However, Axelrod et al. announced that the data on which this concept is based, shows no evidence that it is neither universally adaptive nor widespread. Rather, it is suggested that even if few chemicals have low-dose beneficial effects, it would be unjustifiable to incorporate these effects into risk-assessment, as is argued by Renner (2004) and Calabrese and Baldwin (2003). Experimental studies are mostly based on the use of mature and homogenous animals and therefore results cannot be extrapolated on a heterogeneous population (Axelrod et al., 2004). Opponents of the hormesis concept also postulate that even if the effect seems to be beneficial, it is not necessarily a good thing (Vom Saal, 2007).

*Counterexamples of hormesis:* Negative side-effects are rarely investigated and opponents of the hormesis concept accuse hormesis proponents that they focus on single endpoints such as cancer, while ignoring other endpoints (Kaiser, 2001). For example cyclophosphamide, a cancer drug that stops cell-divisions, protected rats from flu viruses at low doses, but injected with tumor cells, these animals displayed a higher probability to develop cancer (Portier, 1993). Furthermore, endocrine disruptors (hormones and chemicals mimicking hormones), although displaying a biphasic dose-response, contradict the assumptions made by the hormesis concept (Vom Saal, 2007): at high toxicological doses hormones (e.g. estradiol) down-regulate their receptors and bind to other members of the nuclear receptor superfamily. Thus, they induce and inhibit different gene sets in low and high dose treatments; hence, they provoke different biological effects. For example, fetuses or babies exposed to low concentrations of estrogenic chemicals, formerly assumed to display no effect, reveal genetic imprinting with adverse effects on cell functions, which can lead to cancer. For an overview of the controversy and counterexamples of hormesis be referred to Kaiser (2001), Axelrod et al. (2004) and Vom Saal (2007).

*The xenohormesis-concept:* Originated by Lamming et al. (2004) to explain the favorable effect of resveratrol on *Saccharomyces cerevisiae* lifespan, the **Xenohormesis hypothesis** argues that plants which are exposed to stress produce molecules that can prolong the lifespan of the consumer. Resveratrol, a polyphenol built in plants after injury or infection (Langcake and Pryce, 1976) activates Sir2, which may underlie the health benefits of CR. Baur and Sinclair (2008) postulated that to a yeast cell growing on a grape, an increase in the concentration of resveratrol might be a useful indicator that the food supply is coming to a limit. Therefore the yeast cell might gain a selective advantage by responding to resveratrol in the same way it would to an actual deficit of calories, i.e. activation of sirtuin enzymes. Yun et al. (2005) further developed this theory by stating that stressful conditions in modern husbandry and agriculture lead to stressed food, which in turn can act as an illegitimate signal of chronic stress in the consumer and possibly lead to obesity, by erroneously biasing hosts towards caloric accumulation despite the abundance of energy.

However, to date xenohormesis is a more speculative theory, in general suggesting that heterotrophs (animals and fungi) are able to sense chemical cues synthesized by plants and other autotrophs in response to stress. Thereby plants' secondary metabolites ("xeno"- compounds) - produced under stressful conditions like drought, disease and weather - serve as a molecular warning when affiliated by the animal consumer, who in turn is able to prepare itself in prior to

unfavorable conditions. Moreover, the theory predicts that 'xenohormetic' molecules bind to conserved domains in proteins, both, as agonist on one enzyme and as an antagonist against another (Howitz and Sinclair, 2008). This might explain the presumed variety of underlying action mechanisms, which all serve to protect the animal consumer.

## 1.5 Goals of this study

The identification and analysis of compounds that delay aging and extend lifespan is an important aspect of gerontology research. Besides testing theories of aging and the discovery of endogenous systems that influence aging, it establishes the basis for treatments that might delay normal human aging. Therefore, the present study identified three polyphenols (PPs) with lifespan extending and stress-tolerance enhancing properties in *C. elegans*, namely Quercetin (Q), Caffeic acid (CA) and Rosmarinic acid (RA), which belong to the two major subgroups within the PPs, the flavonoids (Q) and the phenolic acids (CA and RA). Investigations of these three PPs allows comparing the effects of PPs belonging to distinct classes on one hand (Q *vs.* CA and RA), and the effect of two structurally close related PPs (CA and RA) on the other. The broadest emphasis in this study was put on Q. Thereby insights were provided, whether PPs act through common or exclusive mechanisms. Furthermore, this study aims to contribute to a better understanding and enlightenment of underlying mechanisms at the physiological and genetic level, by answering following key questions:

**(i) Is the chosen test design adequate and does not produce artifacts in lifespan extension?**

*Are antimicrobial properties of the PPs responsible for life extension?* Proliferating *E. coli* can produce deleterious metabolites for aging *C. elegans* which are grown on the bacterial layer. Antibacterial properties of the PPs could diminish the growth of the food source *E. coli* and thus reduce the negative impact on aged nematodes, hence extending their lifespan. Therefore it is reasonable to check the antimicrobial potential of each PP against *E. coli*.

*Methods:*

- The growth of proliferating *E. coli* was monitored with and without PPs, to discover antibacterial properties.
- The lifespan of wild type *C. elegans* was determined when grown on heat killed OP50 mixed with respective PP from the 6$^{th}$ day of adulthood on, to evaluate the influence of the possible antibacterial properties on the lifespan.

*Is it possible that transgenerational effects of the PPs cause variations in life extension due to different exposure times?* Certain compounds can lead to genetic imprinting (e.g. endocrine disruptors; Vom Saal, 2007) or provoke other epigenetic changes on the DNA, which lead to additive or adverse effects in the offspring of the exposed parental generation. To find out if it is adequate to use F1 nematodes which developed in treated P0 hermaphrodites, and therby exclude associated artifacts, Q treated nematodes have exemplarily been investigated for all three PPs.

*Methods:*

- To gain hints on possible transgenerational effects, lifespan assays with wild type nematodes treated with Q in four different exposure scenarios were conducted: P0 exposed to Q from L4 on, F1 descendent from treated P0 exposed to Q during the whole lifetime, F2 descendent from treated P0 and F1 treated during the whole lifetime, F2 descendent from exposed P0 and F1 but exposure was stopped at L4 and henceforth grown on non-PP-containing plates.

**(ii) Do these three PPs promote longevity via hormesis?**

Since hormesis gains growing interest in the scientific community and a growing proportion of compounds have been identified displaying a hormetic action scheme, all three PPs were evaluated concerning the potential to be classified as hormetins.

*Methods:*

- Performance of lifespan assays over a broad concentration range to identify typical biphasic, inverted J-shaped dose-response curves, characteristic for hormetins.
- Further evidence of the hormetic character by evaluation of the gene expression levels of six *hsp* genes, as a marker for elevated repair responses and thus hormesis action.
- Evaluation of the global transcriptional response in Q treated *C. elegans*, to identify expression changes in genes belonging to repair and biotransformation systems.

**(iii) Do direct or indirect CR effects contribute to lifespan extension?**

CR is the only approach that is accepted to prolong lifespan in a broad range of species. Since the PP resveratrol is suggested to induce sirtuins and thereby activate an indirect CR response, it is conceivable that the PPs investigated in this study also act through this pathway. Furthermore it is possible that direct mechanisms, like the diminished affiliation of food triggered by respective PP - caused by either avoidance of food or a decreased pumping frequency – also lead to a CR response.

*Methods:*

- Performance of lifespan assays with *sir-2.1* mutants, to find a possible lack of response to respective PP.
- An attraction assay was conducted, to find out whether or not nematodes are distracted by the PPs supplement in the bacterial diet and thereby displaying an indirect CR effect.
- Monitoring of the pharyngeal pumping to exclude an alternative cause of CR by decreasing food ingestion.

**(iv) Does re-allocation of resources take place in a disposable soma like manner?**

It is conceivable that the longevity phenotype of Q, CA and RA treated nematodes takes place on the expense of other physiological parameters, e.g. the reproduction capacity, the growth and others. To determine whether or not such reallocation events occur, different experiments were chosen.

*Methods:*

- The reproduction has been examined concerning the total amount of offspring per nematode, and additionally for the time to start egg laying to detect potential developmental delays.
- The growth of the nematodes has been examined and variations between treated and untreated nematodes have been determined.
- Differences in the fat deposits in Lysosome related organells (LROs) have been measured with a Nile Red fluorescence assay to reassess the presumed relationship between a decrease in staining and an enhanced stress-resistance (O'Rourke et al., 2009).

**(v) Are *in vivo* antioxidative properties of the PPs responsible for the longevity effect as proposed by the FRTA?**

Many PPs display antioxidant properties *in vitro*, which were formerly thought to be the only reason for the positive effects of PPs in organisms. Evidence from recent studies, argues that an *in vitro* antioxidant effect is not compulsory associated with *in vivo* antioxidant properties and furthermore is doubtable that the *in vivo* antioxidant property of a given compound is responsible for (all) observed positive effects in the organism (Pun et al., 2010). Thus, it is important to check both, the *in vitro* and *in vivo* antioxidant properties.

*Methods:*

- The total antioxidative capacity (TAC) of each PP was measured in hydrophilic and lipophilic solvents to determine general antioxidative properties *in vitro*.
- The TAC was measured in the hydrophilic and hydrophobic fraction of worm-homogenate, to determine the antioxidative status *in vivo* after treatment with each PP.
- Lifespan assays with *mev-1* mutants, which are hypersensitive to oxidative stress, have been conducted to investigate whether respective PP treatment has the potential to prolong lifespan, which suggests a lowering of internal oxidative stress.
- The intestinal lipofuscin fluorescence – a marker for endogenous oxidative damaged material, also called aging-pigment – was determined, to see a decrease in accumulation, presumably due to slowing of aging.
- Paraquat - a herbicide, commonly used to generate oxidative stress *in vivo* by redox cycling - exposure from L4 on, with concurrent application of respective PP to see a reduction in oxidative stress susceptibility by means of matricidal prevention.

**(vi) Which genetic players are involved in PPs induced longevity and stress-resistance?**

PPs are expected to act through genetic players and signaling cascades to deploy their longevity and stress tolerance enhancing potential. In the past many genes have been identified in *C. elegans* research that have been connected to lifespan and stress resistance.

*Methods:*

- To find such genetic mediators, lifespan assays with mutant strains, lacking genes formerly described as important in lifespan regulation or stress response, have been conducted. For Q treated nematodes 13 strains have been chosen; in CA and RA exposure 8 mutant

strains have been utilized. Presumable players for longevity (no life extension due to PP treatment in respective mutant strain) were confirmed with thermal stress tests.

- To further enlighten the genetic mechanisms, a DNA-microarray assay was performed with three concentrations of Q treated nematodes. In a database search overrepresented GO terms and KEGG pathways have been analyzed. Furthermore, an extensive literature comparison has been conducted in which overrepresented gene groups and gene expression mountains (according to Kim et al., 2001) have been identified. By further matching the Q data to previously published DNA-microarray data from mutants and immune challenged nematodes, the picture of underlying mechanisms of Q mediated longevity shapes clearer.

**(vii) Do Q, CA and RA use common or exclusive mechanisms?**

By comparing findings from Q, CA and RA treated nematodes it is possible to see commonalities or discrepancies between all three PPs. Hence, it is possible to get first insights if structural realted compounds, like these three PPs, share comparable basic action schemes *in vivo* or if they differ considerably.

**(vii) Which current aging theories can be applied to obtained results?**

The obtained results will be discussed in the light of current aging theories. This will in turn help to further understand the processes leading to aging.

# 2 Materials and methods

## 2.1 Strains and growth conditions

All strains were maintained (unless otherwise stated) at 20°C in appropriate incubators on nematode growth medium (NGM) seeded with the *Escherichia coli* feeding strain OP50 according to Brenner (1974). NGM plates were 35 mm or 96 mm in diameter and inoculated with either 100 µl or 1000 µl OP50 ($OD_{595}$ 3.4 - 3.6), respectively. Strains used in this study were: N2, Bristol (wild type); GR1307, *daf-16(mgDf50)*; VC199, *sir-2.1(ok434)*; AM1, *osr-1(rm1)*; AU1, *sek-1(ag1)*; MT2605, *unc-43(n498n1186)*; TK22, *mev-1(kn1)*; DR1572, *daf-2(e1368)*; VC8, *jnk-1(gk7)*; TJ1052, *age-1(hx546)*; DR20, *daf-12(m20)*; EU1, *skn-1(zu67)*; VC204, *akt-2(ok393)*; AE501, *nhr-8(ok186)*. All *C. elegans* strains as well as the OP50 strain were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota, USA. Q (Quercetin dihydrate; ≥ 98%, Fluka NO. 83370 Sigma-Aldrich, Germany), CA (Caffeic acid ≥ 99%, NO. C 0625, Sigma-Aldrich, Germany) and RA (Rosmarinic acid ≥ 97%, Fluka NO. 44699, Sigma -Aldrich, Germany) was dissolved in DMSO (Dimethylsulfoxid; Applichem, Germany) and added (final concentration as denoted in respective section) to the NGM medium and the OP50 bacterial feeding suspension. A final DMSO concentration of 0.3 % (v/v) was maintained in control and polyphenol containing plates.

## 2.2 Life table parameters and other functional investigations

### 2.2.1 Lifespan assays

Lifespan of wild type *C. elegans* was tested in several exposure scenarios. The 1st generation (P0) started at L4 larval stage on either control or treatment plates. Eggs of the 2nd generation (F1) developed in the pre-exposed parent and hatched in the corresponding condition and were exposed during their entire lifetime. This approach was basically chosen for all three polyphenols.

Nematodes of the 3rd generation (F2) exclusively tested for Q, were either used to investigate the presence of additive or adaptive effects of this PP, or transferred (as eggs) onto control plates to determine transgenerational responses. The concentrations of Q, CA and RA were 0, 50, 100, 200 µM, plus 250 µM for Q, and 300 and 600 µM for CA and RA treated wild type nematodes as well as 0, 200 (Q and RA), and 300 µM (CA) for the mutants. The mutant assays were conducted using the concentration that was deemed to be most effective in inducing lifespan extension (in wild type). Blinding of studies was not possible due to the colour of Q and RA, which stain the NGM agar.

For each experimental condition at least two independent trials were performed, each comprising ten small agar plates with at least 100 nematodes. The first day of adulthood was defined as day 1. During the reproductive period, adult nematodes were transferred daily to new treatment plates to avoid overcrowding. After reproduction ceased, transfer occurred every third day, until the impact of aging disallowed handling of the nematodes. Alive and dead animals were scored daily until all had died. Animals were considered dead when no response was observed following a gentle touch by a platinum wire. Nematodes which had escaped the plates or died after internal hatch were subtracted from the total number. All lifespan assays were carried out at 20°C, except where mentioned otherwise. Additional temperatures were 15°C and 25°C for Q treated nematodes. Usually lifespan assays were performed with F1 nematodes, yet, only for *ad*

*libitum* fed wild type worms, treated with Q, from all three generations.

To verify if the PPs acts only via antibacterial effects or as CR mimetics, lifespan assays were also conducted with heat-killed (hk) *E. coli* (30 min at 65°C, according to Gruber et al. 2007) or – just for Q - without a bacterial lawn. To avoid excessive loss, only 6$^{th}$ days old *C. elegans* (F1 generation) were transferred to hk-bacteria or bacteria-free plates, respectively.

### 2.2.2 Reproduction

*Broodsize:* 10 nematodes (L4 larvae of the F1 generation) per concentration were placed onto individual treatment plates. Nematodes were cultivated at 20°C and transferred daily to new plates until reproduction was completed. The total offspring of each individual nematode was counted once grown to L2 or L3 stage.

*Reproduction delays:* Reproduction was assessed by plating nematodes (the P0 generation) onto control or treatment plates (Q 200 µM, CA 300 µM and RA 200 µM) and incubated at 20°C for 4 days. Subsequently, worms were synchronized through egg preparation according to Strange et al. (2007) with sodium hypochlorite (Sigma). Eggs (the F1 generation) were allowed to hatch and arrest at L1 stage by rotating (20 rpm) in M9 buffer over night at room temperature. Ten L1 larvae were transferred to respective control or treatment plates and cultured until adulthood. Approximately 21 h after reproduction commenced, adult nematodes were removed and the number of offspring determined after the nematodes had grown to the third larval stage.

### 2.2.3 Growth alterations

*Volumetric area of living wild type worms from hatching up to day 8*: Changes in the volumetric area (from hatching to day 8) were measured as follows: Synchronized F1 wild type nematodes were obtained as described above and the resultant L1s transferred to treatment plates. To avoid overcrowding, due to reproducing adults, worms were periodically moved to new plates using a platinum wire pick. Photos were taken with a standard upright microscope/camera system (SMZ1500, Nikon UK Ltd., Kingston upon Thames, UK) every 24 h until day 8 (180 h). The volumetric area within the perimeter of the nematodes was measured with Image-Pro Express 5.1 software (Media Cybernetics, Wokingham, UK).

*Length alterations of heat-killed nematodes at the 6$^{th}$ day of adulthood:* The growth assay utilized a minimum of 30 nematodes of the F1 generation per concentration and trial. Nematodes were cultured at 20°C. On the 6$^{th}$ day of adulthood, nematodes were killed by heat exposure (150 minutes/45°C) and the length of each individual nematode was measured using a microscope equipped with a graduated eyepiece.

### 2.2.4 Pharyngeal pumping rate

At the 3rd, 6th and 10th day of adulthood the pharyngeal pumping rate was quantified under a microscope at high magnification. In detail, for every dose and age point, 10 (pre-treated) F1 nematodes were randomly selected and the pumping frequency determined three times over a 15 s time span.

### 2.2.5 Stress resistance

*Thermotolerance:* F1 generation nematodes were cultured as described above for lifespan assays and kept at 20°C on either control (0 µM) or PP (Q 200 µM, CA 300 µM, RA 200 µM) plates until the 6th day of adulthood. The thermal resistance test was performed with approximately 120 nematodes for each concentration in each trial. At the 6th day of adulthood, plates were switched to 35°C. After 8 h for wild type N2 and *unc-43(n498n1186)*, 9 h for *age-1(hx546)*, 10 h for *daf-2(e1368)*, 7 h for *sir-2.1(ok434)*, *osr-1(rm1)* and *daf-16(mgDf50)*, and 6 h for *sek-1(ag1)* dead and alive nematodes were counted.

*Oxidative stress resistance with 10 mM paraquat:* Wild type *C. elegans* (L4 stage) were placed on control and treatment plates (0 µM, Q 200 µM, CA 300 µM, RA 200 µM) in the presence of 10 mM paraquat (NN-dimethyl-44´-bipyridinium dichloride, ABCR GmbH & Co. KG, Karlsruhe, Germany) added to the agar and the OP50 food source. Survivors were counted after 72 h.

### 2.2.6 Attraction assay

NGM agar plates (92 mm) were prepared containing six alternating spots of bacteria around the peripheral region of the plate, lacking or supplemented respective PP (Q 200 µM, CA 300 µM, RA 200 µM). The bacterial spots on the agar surface were identical in size and quantity. Immediately after air-drying one L4 juvenile F1 worm was placed on each of the six spots, incubated at 20°C for 24 h, and thereafter the number of next generation (F2) offspring on each individual spot was determined. The combined number of total F2 offspring present on the three control spots was set to 100% and compared to the F1 offspring counted on the three spots supplemented with PPs.

### 2.2.7 Bacterial growth assay

*E. coli* OP50 bacteria ($OD_{595}$ = 0.2) were spiked with 0.3% (v/v) DMSO, Q 200 µM, CA 300 µM or RA 200 µM and the $OD_{595}$ at this time point taken as the blank at time point zero. Tubes were incubated at 37°C on a shaker (240 rpm) and the $OD_{595}$ was measured every 60 min until all samples reached an OD in excess of 1.0.

### 2.2.8 Fluorescence measurements

Synchronized wild type (F1 generation) nematodes were prepared by egg preparation as described above. L1 larvae were transferred to control and treatment plates inoculated with OP50. To avoid overcrowding, worms were transferred daily during the reproductive period. On day 3 and 6 of adulthood, 10-20 worms per condition (0 µM, Q 200 µM, CA 300 µM, RA 200 µM) were placed on microscope slides, prepared with 2% agarose pads and immobilized with 50 mM sodium azide (Sigma). The mean fluorescence intensity was quantified using Image ProExpress by determining the area of nematodes and the sum of fluorescence.

*Nile red staining of intestinal fat deposits*: OP50 was supplemented with Nile red to a final concentration of 6 µM. Pictures were taken at the 3rd and 6th day of adulthood. Individual nematodes were observed and photographed using an inverted fluorescence microscope with a 200x magnification (Nikon TE2000S).

*Intestinal lipofuscin fluorescence:* Individual nematodes were observed and photographed at 100x magnification fluorescence microscope (Nikon Eclipse E200). Pictures were taken at the 7th day of adulthood.

### 2.2.9 Total antioxidative capacity (TAC)

For each condition (0 µM, Q 200 µM, CA 300, RA 200 µM) 40 staged L4 wild type *C. elegans* were grown on three 96 mm agar dishes (inoculated with 1 ml bacterial suspension) for 4 days, after which nematodes were rinsed off and washed with M9 buffer. The resultant worm pellets were homogenized in a "SpeedMill" (Analytik Jena, Germany). Sample disintegration was achieved by ten cycles of rapid shaking (30 s) followed by ice-cooling (1 min). The homogenate was centrifuged and the supernatant used to determine the antioxidative capacity of both aqueous- and lipid-soluble substances. Therefore, a PHOTOCHEM® (Analytik Jena, Germany) driven photochemiluminescence (PCL) approach (Popov and Lewin 1999) was applied to define the TACs of pure PP standard solutions.

The results are presented in equivalent concentration units of ascorbic acid (AA) for water-soluble substances or Trolox® (T) units for lipid-soluble substances. The PCL assay combines the fast photochemical radical generation with the sensitive luminometric detection. This reaction is induced by optical excitation of a photosensitizer which results in the generation of the superoxide anion radical $^{\bullet}O_2^-$. These radicals are partially eliminated from the sample by reaction with the antioxidants present in the sample. In the measuring cell of the PHOTOCHEM® the remaining radicals cause the detector substance to luminescence. For more details, refer to the original description of Popov and Lewin (1999). The antioxidant capacity of the sample is determined in a separate cell by means of a photomultiplier tube. The hydrophilic and lipophilic antioxidants were quantified using the ACW (by means of the lag phase for water-soluble water-soluble substances) and ACL (by means of the area under the curve for lipid-soluble substances) kits (Analytik Jena, Germany), respectively. The preceding lipid extraction was performed according to Bligh and Dyer (1959) and the measurements of the corresponding protein via a standard Bradford assay (Bradford, 1976).

According to previous experiences with multiply challenged waterfleas (Steinberg et al. 2010), total antioxidant capacity of organisms reflects the deviation of the redox homeostasis more accurately than the sum of single antioxidants or the modulation of single antioxidant enzymes. These experiences were based on the gaschromatographic monitoring of ethylene development from a-keto-c-methiol-butyric acid, which is inhibited in the presence of antioxidants. Given that the method applied here exploits photochemiluminescence, it should be more sensitive (by a factor of at least $10^2$) than any previously applied detection.

### 2.2.10 Data interpretation and statistical analysis

All tests comprised of at least two - mostly more - independent experimental trials. Alterations in lifespan values of treated versus untreated nematodes were specified in percentage, both for mean and median lifespan. Statistical significance was calculated by means of the log rank test (Bioinformatics at the Walter and Eliza Hall Institute of Medical Research; http://bioinf.wehi.edu.au/software/russell/logrank/). Mean values were also calculated for reproduction, body length, growth rate alterations, Nile red staining, bacterial growth, *hsp*-levels and TAC, respectively. Statistical significance was evaluated by one way ANOVA (Sigma Stat 3.5, SPSS Inc., USA). For the thermotolerance and oxidative stress assay (paraquat), mean survival rates were calculated. Statistical significance in the thermotolerance, oxidative stress and attraction assays were identified by the Chi Square Test (Sigma Stat 3.5, SPSS Inc., USA). Variances were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.005$.

## 2.3 Molecularbiological experiments

### 2.3.1 Expression levels of heat shock protein genes (*hsps*) quantified by reverse transcriptase –PCR (qRT-PCR)

*Sample generation:* For qRT-PCR synchronized first-staged wild type animals were grown at 20°C until the 1$^{st}$ day of adulthood at a density of about 500 animals/plate (90 mm in diameter), in the presence of either 0 µM PP, 200 µM Q, 300 µM CA or 200 µM RA. Animals were removed from plates with a platinum wired worm pick, transferred into micro-centrifuge tubes with cold M9 buffer, allowed to settle on ice, then shock frozen in liquid nitrogen and stored at -80°C. For each condition worm samples were collected in duplicates.

*RNA preparation and cDNA synthese:* The packed frozen worm samples were disrupted, using 0.5 mm glass beads, in a SpeedMill homogenizer (Analytik Jena). Two 2 min cycles of shaking, were followed by 5 min of cooling on ice. RNA was prepared according to the TRizol® extraction protocol by Chomczynski and Sacchi (1987) with few modifications. After further purification with Chloroform and Isopropanol (Roth, Karlsruhe, Germany) the RNA was precipitated with ethanol and resuspended in sterile water. The cDNA synthesis was performed with M-MLV revertase (Promega, Madison, USA).

*RT-PCR:* Real-time PCR was conducted in the MyiQ5 System (Bio-Rad, USA) using the fluorescence dye EvaGreen (Bioscience, Jena, Germany) and green core qPCR polymerase (Jena Bioscience GmbH, Germany), 2 µl cDNA and 100 µM primers (Primerdesign is shown in Table 1). Signals were normalized using the reference gene beta-actin (*act-1*) as an invariant internal control. The relative expression of the target genes has been calculated using the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Following gene-specific primers were used:

*Data interpretation and statistical analysis:* Mean values were calculated. Statistical significance was evaluated by one way ANOVA (Sigma Stat 3.5, SPSS Inc., USA).

**Table 1: Primersequences** for *hsp*-gene expression measurement

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| *act-1* (T04C12.6) | TCCAAGAGAGGTATCCTTAC | CGGTTAGCCTTTGGATTGAG |
| *hsp-3* (C15H9.6) | CCGTCACCATCCAGGTCTTC | GGTTCCCTTATCCTCGGCAG |
| *hsp-12.6* (F38E11.2) | AGTTGTCAATGTCCTCGACG | CTTCAATGTGAAGAATTCCATGTG |
| *hsp-16.1* (T27E4.8) | CTGAATCTTCTGAGATTGTTAAC | TTTGTTCAACGGGCGCTTGC |
| *hsp-16.41* (Y46H3 A2) | ATTGGGGAGATTGTAAATGATG | GCGTTTCAAGTATCCATGTTCC |
| *hsp-17* (F52E1.7) | ATGGCCGATTCTTCGATGAC | GGACAAAGTGACGCTCTACC |
| *hsp-70* (F44E5.4) | GAAAGGTTGAGATCCTCGCC | CATCGAAACGTCGTCCAATC |

### 2.3.2 DNA-microarray analyses

*Sample generation:* Untreated P0 nematodes were chunked out on control and treatment plates (96 mm in diameter; 0 µM, Q 50 µM, Q 100 µM, Q 200 µM) and incubated at 20°C for 4 days. A synchronous culture was generated by rinsing F1 nematodes from Q containing plates and subsequently filtered through a 10 µm pore size membrane filter (SM 16510/11, Sartorius, Germany), which retains all but first-staged juveniles (L1). After filtering L1 nematodes were put again on treatment plates. Worms were grown to the young adult stage, harvested by rinsing off with M9 buffer and kept on ice until freezing in liquid nitrogen and stored at -80°C. Each condition was collected in triplicates.

*RNA preparation and conduction of DNA-microarray analyses:* The packed frozen worm samples were disrupted by using a SpeedMill homogenizer (Analytik Jena) and special metal oxide beads in disintegration tubes. 2 min shaking followed by 5 min on ice was repeated twice to disintegrate samples. Subsequently the whole RNA was purified utilizing a Total RNA Isolation kit from Macherey-Nagel including a DNase step to remove genomic DNA. Samples were stored at -80°C until usage. Further procession of RNA was performed with MessageAMP$^{TM}$ Premier RNA Amplification Kit (Ambion). RNA sample preparation is based on the T7 in vitro transcription (IVT) amplification technology, also known as reverse transcription-IVT (RT-IVT) method (Van Gelder et al., 1990). First- and Second-Strand cDNA synthesis, aRNA synthesis, labeling, fragmentation, GeneChip hybridization, and scanning were performed according to specifications from Affymetrix. Triplicate chips were run for each condition (0, Q 50, Q 100, Q 200 µM). 22548 transcripts were abundant on the chip. RNA and cDNA qualities were verified at each step using capillary electrophoresis (Bioanalyzer; Agilent Technologies). Quantities were measured photometrically (NanoDrop ND-1000 spectrophotometer, Thermo Fisher Scientific, Wilmington, DE, USA).

*Data interpretation and statistical analysis of DNA-microarray data:* Pre-processing of DNA-microarray raw data included probe-specific background correction, summarization of probe set values, and normalization using the GCRMA algorithm with CARMAweb 1.4, an R- and Bioconductor-based web service for DNA-microarray data analysis (Rainer et al., 2006). Quality of the normalization was accessed by boxplot, and MA-graph analyses. Differences between treatments were visualized by Principal Component Analysis (PCA) plotting with MeV (Saeed et al., 2003). Data were initially filtered out for missing values and then subjected to a CLEAR-test that combines differential expression and variability using the GEPAS web server at http://www.gepas.org (Tárraga et al., 2008). The differential expressions of genes between the treatment group vs. reference control were compared using unpaired t-tests and significance analysis of microarray (SAM) testing; the latter includes a calculation that estimates the false discovery rate (FDR). Here the false discovery rate was set to a non-stringent level of <12.5%.

Differential expressed genes (DEGs) showing a fold change of 1.25 were further analyzed with regard to their molecular functions, biological processes, and biological pathways using information provided by Wormbase (www.wormbase.org) with high throughput GoMiner software (Zeeberg et al., 2005) and DAVID (http://david.abcc.ncifcrf.gov/).

*Representation Factor:* As measurement of overlap between differing conditions (e. g. PPs and diverse gene expression mountains, gene groups or datasets in the literature), the Representation Factor (RF) was taken to definite the fold enrichment. It shows whether genes from one list (list A) are enriched in another list (list B). (Definition of RF: (number of genes in common between both lists)*(number of genes in the genome)/(number of genes in list A)*(number of genes in list B)). The choice of N (genome) was based on the values recommended by the authors. Intersection p-values were calculated from the hypergeometric distribution. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$

# 3 Results

## 3.1 Possible artifacts: antimicrobial properties and transgenerational effects

### 3.1.1 Q and RA dimish the bacterial growth of *E. coli*

Given that some PPs possess antimicrobial properties, it was deemed important to investigate to what extent Q, RA and CA influence the growth of the gram-negative *E. coli* feeding strain OP50 and thereby cause advantages for life extension. In contrast to CA, which did not inhibit bacterial growth, RA and in particular Q both exerted significant effects over the 4.5 h cultivation period (Fig. 7).

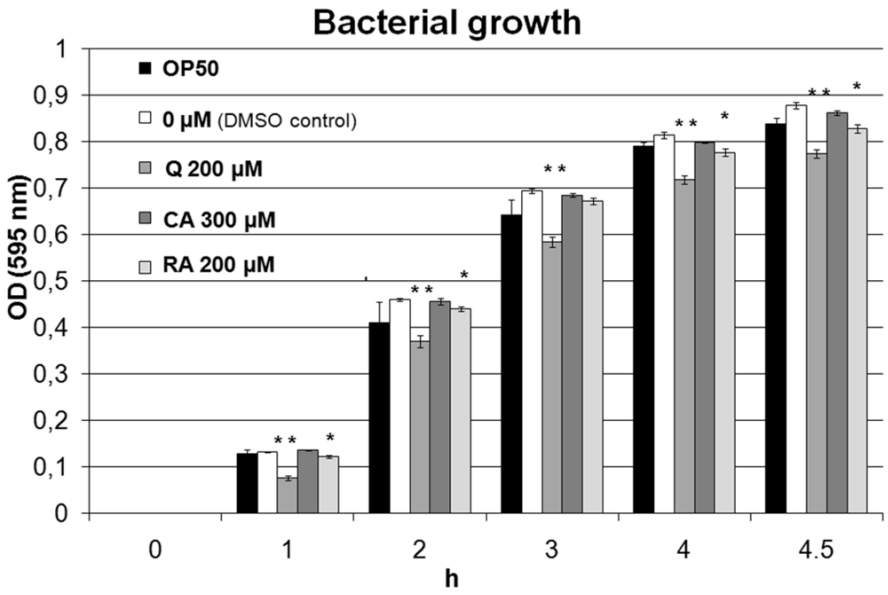



**Fig. 7 Antibacterial properties.** Bacterial growth test with pure OP50, OP50 containing 0.3% DMSO (0 µM, control), or OP50 with 200 µM Q, 300 µM CA or 200 µM RA dissolved in DMSO. Graphs combine three biological replicates. Bars represent SEM. *p < 0.05, **p < 0.005 (One Way Anova).

### 3.1.2 Growing on heat-killed *E. coli* does not prevent life extension

To investigate if these antibacterial properties are linked to the observed lifespan extension, further assays were conducted with heat-killed bacteria. However, even after being fed with dead OP50 mixed with Q, CA or RA, nematodes exhibited PP induced average lifespan extensions of 9%, 15 % and 22 %, respectively (Fig. 8 A, B, C). Thus, the hypothesis that longevity may be due to antibacterial-action of PPs was rejected. (Additional lifespan data for each individual experimental trial can be found in Tables 3, 4 and 5.)

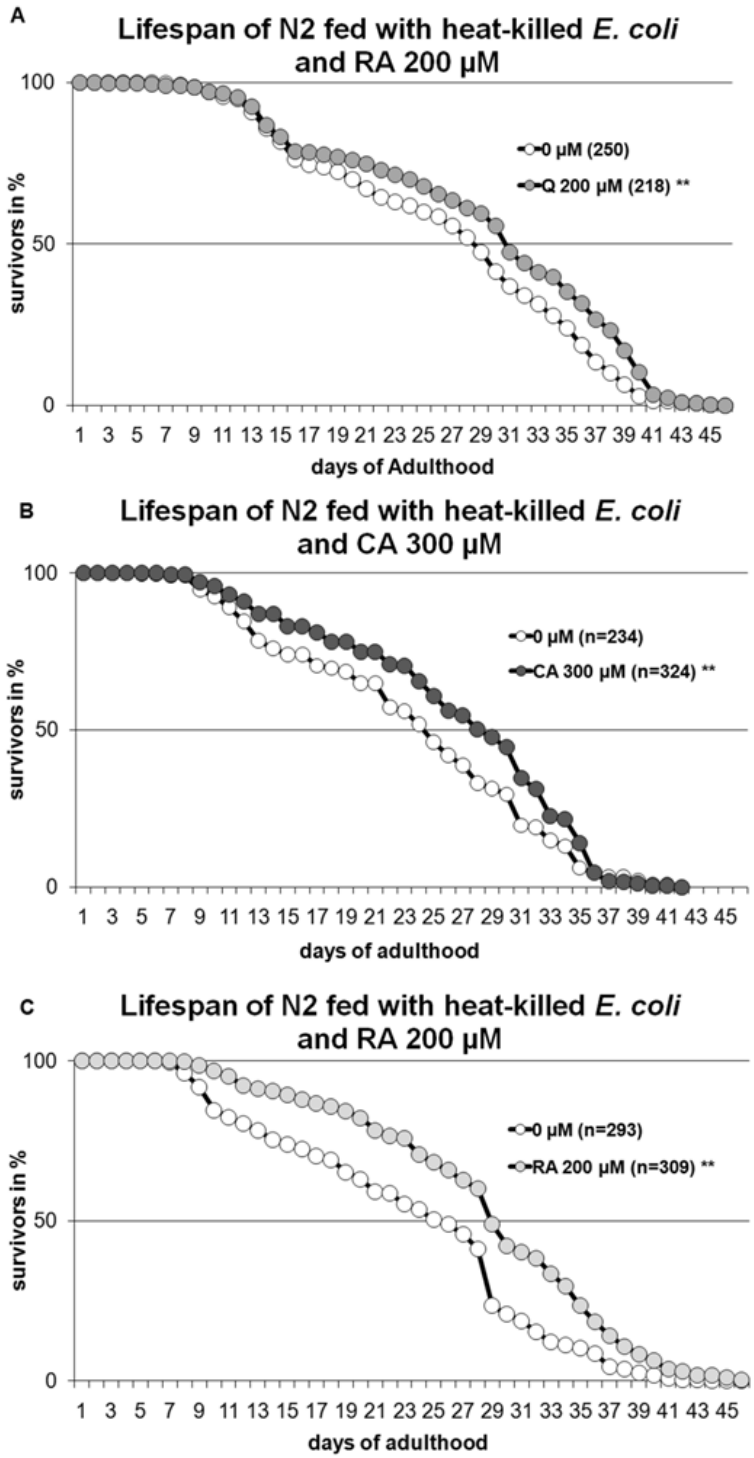



**Fig. 8 Lifespan of wild type nematodes fed with heat-killed *E.coli* and Q 200 µM (A), CA 300 µM (B) or RA 200 µM (C).** Detailed mean and median lifespan data, n and trial numbers, as well as single trial significances are listed in Tables 3 (Q), 4 (CA), 5 (RA). Bars represent SEM. *p < 0.05, **p < 0.005 (log-rank test).

### 3.1.3 Transgenerational effects of Q treatment are negligible

It was also important to check, whether PPs could have adaptive or additive transgenerational effects which also provoke artifacts in life-extension, to find out if it is adequate to use whole life-time PP-treated F1 nematodes for following experiments. To get first hints if PP treatments have the potential to cause types of epigenetic, additive or adaptive changes on lifespan throughout

generations, an experiment was conducted to determine (i) if the treatment through generations causes yet increased or diminished results, or (ii) if the lifespan of the untreated offspring of PP exposed worms is also enhanced. These tests are exemplarily for PPs performed with Q treated nematodes.

Q enhanced lifespan in all generations tested (Fig. 9 A – C), an effect that was statistically significant in the F1 and F2 generation, but not in P0. This may be a result of differing exposure times (the P0 generation was exposed only from L4 stage onwards) or an effect triggered during embryonic development, a question that clearly warrants further investigations. Nevertheless, lifespan extension was modulated in a Q dose responsive manner. In detail, the mean lifespan of the F1 generation was prolonged by 11 and 18% in the presence of Q 100 and Q 200 µM, respectively (Table 2). The median lifespan was expanded by up to 21% (Table 2). The experimental setup was purposefully designed to test for additive (mean lifespan of F2 > mean lifespan of F1) or adaptive effects (mean lifespan of F2 < mean lifespan of F1). Although the first experimental trials suggested that lifespan was extended in the F1 generation (compared to F2), further seven experimental trials could not confirm this perceived trend (compare Table 3). However, life extension of the F1 and F2 generation were comparable (Fig. 9 B and C, Table 2). Overall this suggests that Q displays no additive or adaptive effects in lifespan regulation. Similarly, no beneficial transgenerational impact were observed, i.e., when the ancestor (P0 and F1) but not the offspring generation (F2) is treated with Q (Fig. 9 D, Table 2). These results justify the use of F1 nematodes in the other experiments.

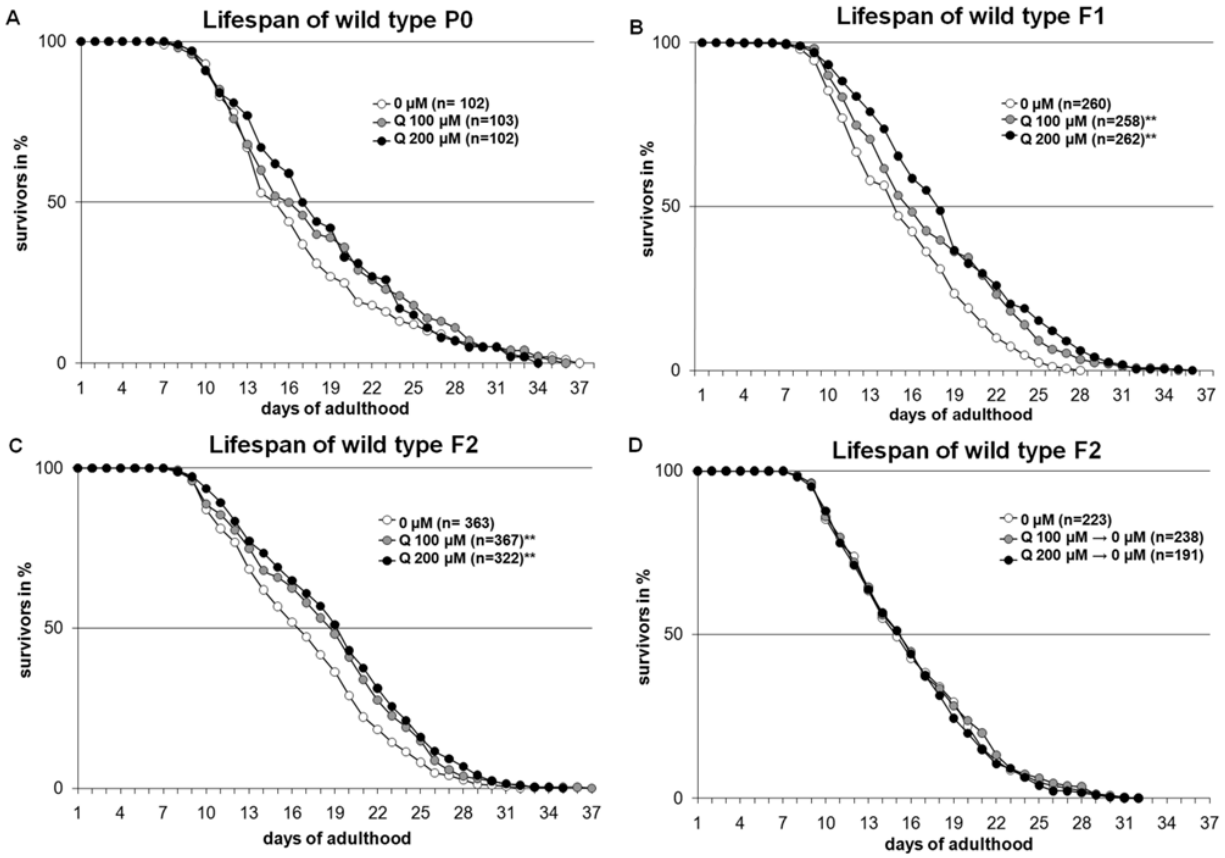



**Fig. 9 Possible transgenerational effects.** Q significantly extends the lifespan in *C. elegans*, shown are survival curves of nematodes of the first (P0) (A), second (F1) (B) and third (F2) (C) generation exposed to 0, 100, and 200 µM Q, respectively. Q treatment did not show transgenerational effects in the F2 generation, when nematodes were replaced to non-treatment plates as eggs (D). Graphs combine 2–3 independent trials, for further details see Table 2, *p < 0.05, **p < 0.005 (log-rank test).

**Table 2 Median and mean lifespan of Q treated wild type *C. elegans* in different application scenarios.** Refers to Fig. 9.

| Q (µM) | Generation | Median lifespan (d) ± SEM | Change ± SEM | Mean lifespan (d) ± SEM | Change ± SEM | n | Trials | Change in single trials |
|---|---|---|---|---|---|---|---|---|
| 0 | | 14.90±1.03 | | 17.10±0.72 | | 102 | | |
| 100 | P0 | 16.60±0.38 | 1.11±0.07 | 18.40±0.16 | 1.08±0.04 | 103 | 2 | 1.13, 1.03 |
| 200 | | 17.70±0.54 | 1.19±0.17 | 18.70±0.10 | 1.09*±0.05 | 102 | | 1.15*, 1.05 |
| 0 | | 14.69±1.61 | | 15.72±0.70 | | 260 | | |
| 100 | F1 | 15.68±0.29 | 1.07±0.10 | 17.47±0.03 | 1.11**±0.06 | 258 | 2 | 1.06*, 1.17** |
| 200 | | 17.79±0.30 | 1.21±0.12 | 18.54±0.15 | 1.18**±0.08 | 262 | | 1.10**, 1.25** |
| 0 | | 16.40±2.05 | | 17.11±1.22 | | 363 | | |
| 100 | F2 | 18.64±1.59 | 1.14±0.06 | 16.73±1.11 | 1.08**±0.03 | 367 | 3 | 1.05, 1.08*, 1.13** |
| 200 | | 19.39±2.50 | 1.18±0.04 | 19.05±1.54 | 1.11**±0.03 | 322 | | 1.07, 1.14**, 1.11* |
| 0 | | 14.90±1.80 | | 16.15±1.05 | | 223 | | |
| 100→0 | F2 | 15.18±1.43 | 1.02±0.03 | 16.40±0.92 | 1.01±0.01 | 238 | 2 | 1.01, 1.03 |
| 200→0 | | 15.17±1.46 | 1.02±0.03 | 16.09±0.74 | 1.00±0.02 | 191 | | 0.97, 1.02 |

Statistical significance was calculated by log-rank test, changes in mean lifespan considered significant at *p < 0.05 or **p< 0.005

## 3.2 Hormetic working modes

### 3.2.1 PPs display concentration-dependent lifespan extension

All three PPs exert a hormetic dose response that is defined by an inverted U-shaped curve (Fig. 10). Whilst 50 µM of Q evoked no significant change in life extension, 100 and 200 µM Q exerted an effect that was reversed at 250 µM Q (for more details see Table 3). Similarly, exposure to 100 µM to 600 µM (but not 50 µM) of CA was found to significantly extend the lifespan of wild type *C. elegans* (Table 4). Indeed, the strongest effect was observed at 300 µM CA (11 % life extension). For RA, a significant life extension was induced at 200 µM and 300 µM RA, but notably not at 50 µM and 100 µM RA. Nematodes treated with 600 µM RA resulted in a significant reduction in lifespan (Table 5). Based on these results, optimal concentrations for maximal lifespan extension were deemed to be 200 µM Q, 300 µM CA and 200 µM RA, thus these concentrations were used in all other experiments.

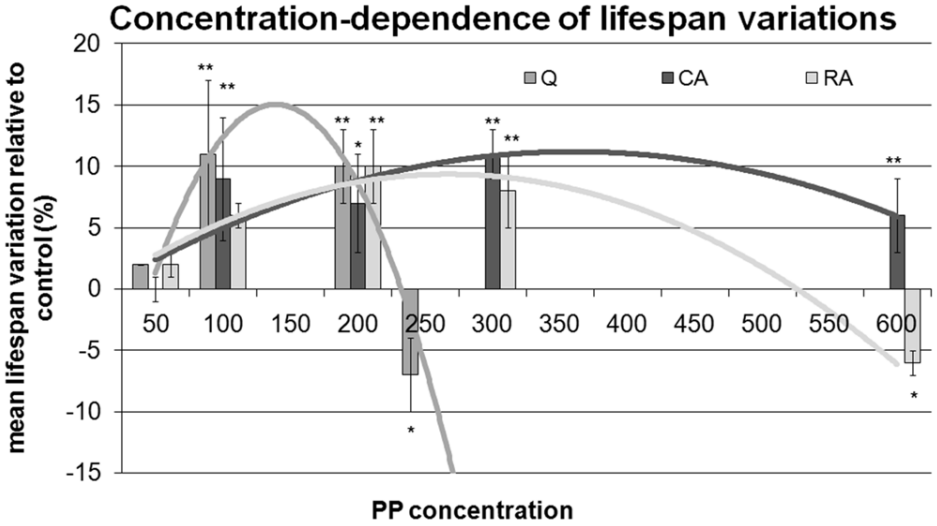



**Fig 10 Lifespan alterations in wild type nematodes treated with variable concentrations of Q, CA and RA.** Shown are the mean lifespan variations in each condition compared to control. Appropriate data (mean and median lifespan, n and trial numbers, single trial significances) are listed in Tables 3, 4 and 5. Lines represent the inverted U-shaped dose-response relationships. Error bars represent SEM, *p < 0.05 and **p < 0.005 (log-rank test).

**Table 3 Median and mean lifespan of wild type *C. elegans* after treatment with differing Q concentrations** (50, 100, 200, 250 µM) **and two alternative feeding scenarios** (heat killed OP50 and without OP50). Refers to Fig. 8 A and 10.

| Concentration Q (µM) | Median lifespan (d) ± SEM | | Change ± SEM | Mean lifespan (d) ± SEM | | Change ± SEM | n Control/ Treated | Trials | Mean lifspan change single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Treated | | Control | Treated | | | | |
| 50 | 16.20±0.40 | 16.79±0.29 | 1.04±0.01 | 16.45±0.62 | 16.84±0.63 | 1.02±0.01 | 186/180 | 2 | 1.02, 1.03 |
| 100 | 14.69±0.78 | 15.68±0.12 | 1.07±0.06 | 15.72±0.70 | 17.47±0.03 | 1.11**±0.06 | 260/258 | 2 | 1.06, 1.17** |
| 200 | 16.09±0.72 | 17.64±0.77 | 1.10±0.04 | 16.43±0.77 | 18.05±0.84 | 1.10*±0.03 | 806/865 | 7 | 1.07*, 1.25**, 1.04, 1.07, 1.12**, 1.03, 1.06* |
| 250 | 17.20±0.13 | 16.11±0.94 | 0.94±0.06 | 17.22±0.05 | 16.04±0.50 | 0.93*±0.03 | 196/171 | 2 | 0.90*, 0.96 |
| 200[a] | 28.45±1.08 | 30.68±2.27 | 1.08±0.04 | 26.57±1.35 | 28.76±1.33 | 1.09**±0.58 | 250/218 | 2 | 1.09, 1.08* |
| 200[b] | 23.80±4.22 | 27.93±4.67 | 1.17±0.08 | 28.91±7.13 | 32.24±9.31 | 1.10**±0.03 | 271/236 | 3 | 1.16**, 1.05, 1.09* |

[a] fed with heat-killed *E. coli* bacteria from the 6th day of adulthood
[b] no food from the 6th day of adulthood (food deprivation)
* $p < 0.05$
** $p < 0.005$
all experiments are conducted at 20°C

**Table 4 Median and mean lifespan of wild type *C. elegans* after treatment with differing CA concentrations** (50, 100, 200, 300, 600 µM) **and an alternative feeding scenario** (heat killed OP50). Refers o Fig. 8 B and 10.

| Concentration CA (µM) | Median lifespan (d) ± SEM | | Change ± SEM | Mean lifespan (d) ± SEM | | Change ± SEM | n Control/ Treated | Trials | Mean lifespan change single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Treated | | Control | Treated | | | | |
| 50 | 17.19±0.14 | 17.27±0.34 | 1.00±0.01 | 17.22±0.05 | 17.22±0.11 | 1.00±0.01 | 169/225 | 2 | 1.00, 0.99 |
| 100 | 13.26±1.70 | 14.64±0.64 | 1.10±0.05 | 14.07±1.44 | 15.30±0.85 | 1.09**±0.05 | 235/261 | 2 | 1.14**, 1.05 |
| 200 | 13.26±1.70 | 13.94±1.34 | 1.05±0.03 | 14.07±1.44 | 15.08±1.01 | 1.07*±0.04 | 235/239 | 2 | 1.04, 1.10* |
| 300 | 13.56±0.79 | 15.39±0.94 | 1.13±0.03 | 14.43±0.76 | 16.08±0.94 | 1.11**±0.02 | 725/710 | 6 | 1.10**, 1.09*, 1.13**, 1.03, 1.15**, 1.19** |
| 600 | 18.20±0.13 | 19.46±0.33 | 1.07±0.03 | 17.22±0.05 | 18.20±0.40 | 1.06**±0.03 | 169/162 | 2 | 1.03, 1.08* |
| 300[a] | 24.22±3.15 | 27.79±2.80 | 1.15±0.03 | 24.35±3.46 | 27.95±3.30 | 1.15**±0.03 | 234/324 | 2 | 1.12**, 1.18** |

[a] fed with heat killed bacteria from the 6th day of adulthood
* $p < 0.05$
** $p < 0.005$
all experiments are conducted at 20°C

**Table 5 Median and mean lifespan of wild type *C. elegans* after treatment with differing RA concentrations** (50, 100, 200, 300, 600 µM) **and an alternative feeding scenario** (heat killed OP50). Refers to Fig. 8 C and 10.

| Concen-tration RA (µM) | Median lifespan (d) ± SEM | | Change ± SEM | Mean lifespan (d) ± SEM | | Change ± SEM | n Control/ Treated | Trias | Mean lifespan change single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Treated | | Control | Treated | | | | |
| 50 | 17.20±0.13 | 17.21±0.04 | 1.00±0.01 | 17.22±0.05 | 17.51±0.16 | 1.02±0.01 | 169/150 | 2 | 1.02, 1.01 |
| 100 | 14.98±2.77 | 16.74±3.89 | 1.12±0.06 | 15.60±3.54 | 16.56±3.75 | 1.06±0.01 | 208/256 | 2 | 1.08**, 1.04 |
| 200 | 15.21±1.27 | 16.86±1.76 | 1.11±0.04 | 15.80±1.17 | 17.45±1.52 | 1.10**±0.03 | 649/654 | 5 | 1.19**, 1.10* 1.13** 1.05, 1.04, |
| 300 | 14.75±2.25 | 16.67±2.76 | 1.13±1.48 | 15.70±1.56 | 17.03±2.14 | 1.08**±0.03 | 238/261 | 2 | 1.11** 1.05 |
| 600 | 17.20±0.13 | 15.81±0.19 | 0.92±0.02 | 17.22±0.05 | 16.14±0.20 | 0.94*±0.01 | 169/208 | 2 | 0.95, 0.92* |
| 200[a] | 25.34±1.00 | 29.41±0.42 | 1.16±0.06 | 22.84±1.89 | 27.84±2.47 | 1.22**±0.02 | 293/309 | 3 | 1.25**, 1.21**, 1.18 |

[a] fed with heat killed bacteria from 6th day of adulthood
*p < 0.05
**p < 0.005
all experiments are conducted at 20°C

### 3.2.2 Induction of *hsp* expression levels is variable

As a marker for elevated repair response and thus for hormetic action, the expression levels of six *hsp*-genes were measured with qRT-PCR. All 6 *hsp*-genes investigated are highly up-regulated following an exposure to RA. Whilst Q treatment was able to induce 4 of 6 *hsp*s, CA resulted in a down-regulation of five *hsp*s and only *hsp-12.6* was upregulated (Fig. 11). It should be noted that although the trends are apparent, not all results are statistically significant (due to the small sample size of just two biological replicates, each with two technical repeats).

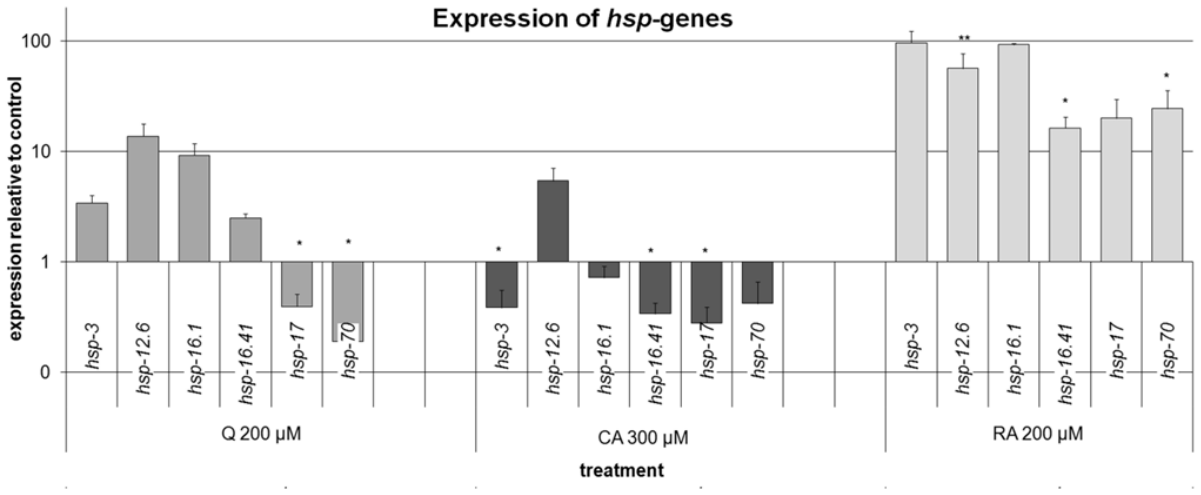



**Fig. 11 Expression of six *hsp*-genes in PP treated nematodes on the 1st day of adulthood.** Columns combine two biological and two technical repeats. Bars represent the positive SEM, controls are set to 1. *p < 0.05 and **p < 0.005 (One Way Anova).

## 3.3 Direct or indirect CR effects

### 3.3.1 PPs enhance the pharyngeal pumping rate throughout the life

To preclude the possibility that the observed longevity is the result of reduced food intake (stimulated by Q, CA or RA exposure), pharyngeal pumping was quantified at the 3rd, 6th and 10th day of adulthood (Fig. 12 A). The pumping frequency declined over time in all conditions, remarkable, however is the highly significant increase in pumping due to PP exposure (in particular Q). Taken together, these findings provide strong evidence that PPs increase food ingestion, thus calorie restriction is unlikely to be the cause of the observed longevity.

### 3.3.2 *C. elegans* are not attracted nor repulsed by PPs

If nematodes are repulsed by the PPs and hence avoid the bacterial food source it is conceivable that the overall food uptake decreases which would provoke a direct CR effect. A behavioral assay revealed the presence of a slight, but statistically non-significant, avoidance response towards Q and RA (control spots/spots with compound [%]: 100/84 for both PPs). The results from CA treatment (100/93) were similar to controls (100/94). Therefore, PPs seem to be, at large neither chemoattractive nor chemo-repulsive (Fig. 12 B).

### 3.3.3 *sir-2.1* lifespan can not be extended by CA and RA

Hints towards indirect CR brought tests with *sir-2.1* mutants. The *sir-2.1* gene, which in the past has been suggested to mediate a genetic CR pathway (Lin et al., 2000; Rogina & Helfand, 2004; Wood et al., 2004; Chen et al., 2005; Viswanathan et al., 2005; Wang and Tissenbaum, 2006) – was identified in mutant lifespan and thermal resistance tests as a genetic player of CA and RA mediated longevity and thermal resistance (CA: Fig. 22 A, 23 A; Tables 8, 10; RA: Fig. 22 B, 23 B; Tables 9,11). Hence it is conceivable that both PPs provoke an indirect CR effect, by activation of this *sir-2.1* mediated CR response (for results and discussion see sections 3.6.2 and 4.6.2). In the case of Q, lifespan assays with *sir-2.1* mutants revealed that Q could extend the lifespan even in the absence of *sir-2.1*, and hence an indirect CR effect was excluded (Fig. 14, Table 4). Further confirmation brought lifespan assays with wild type nematodes, grown on agar plates provided with Q, but without food from the 6th day of adulthood (to avoid excessive loss of worms due to “bagging”) on. If Q would provoke an indirect genetic CR program, it is suggested that no further extension of lifespan would be achieved by this test design. Since the gain in lifespan was significantly enhanced, indirect CR was excluded for Q action (Table 3).

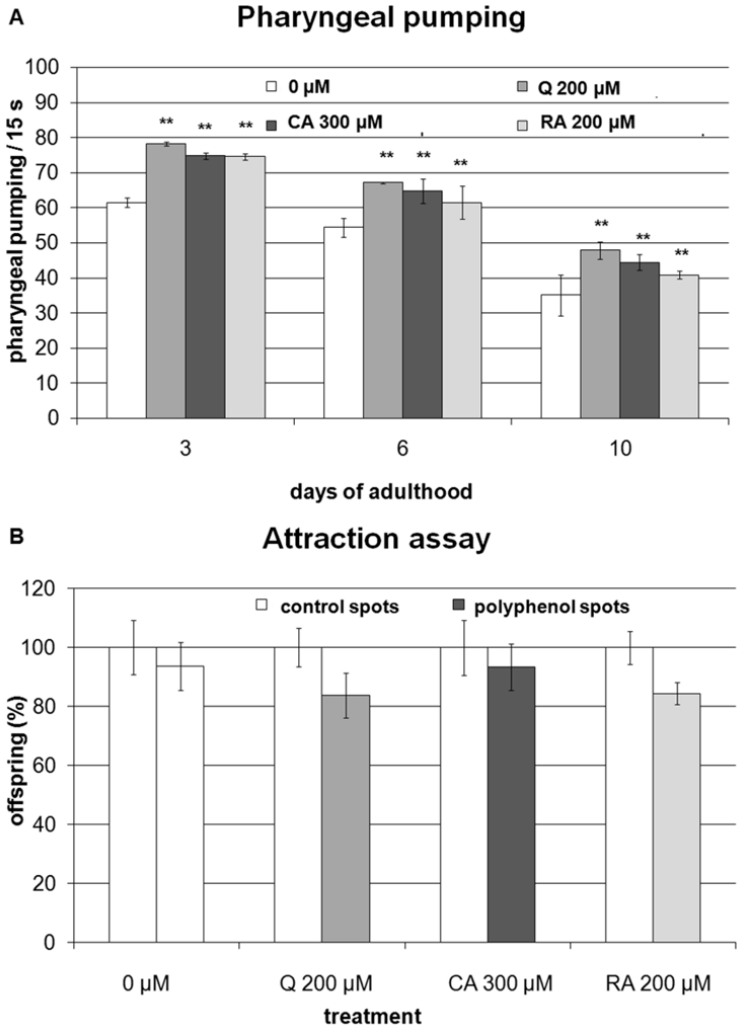



**Fig. 12 Direct dietary restriction effects. (A)** Influence of PPs on the average pharyngeal pumping rate. Shown are the compiled graphs of two single trials (20 worms per condition). Pumping activity was monitored at the 3rd, 6th and 10th day of adulthood; **(B)** PPs in the bacterial diet does not attract nor distract the nematodes. Shown are eight repeats, each repeat was conducted with n= 6 nematodes in each treatment. Wild type *C. elegans* were used for both tests. Error bars represent the SEM. *p < 0.05, **p < 0.005 (Chi Square test **(A)**, One Way Anova **(B)**).

## 3.4 Re-allocation of resources

### 3.4.1 Reproduction: unchanged broodsize but delays in timing

Worms produced on average 301 juveniles in (non-exposed) control conditions and 297, 285 and 298 offspring when exposed to Q, CA or RA (Fig. 13 A). These statistically insignificant differences in the total broodsize are in contrast to the observed drift in reproduction on days 2 and 4 (Fig. 13 A), where CA and RA caused significant variations in offspring numbers, respectively. These results could be underlined by the finding of delayed onset of reproduction, which was significant in the case of RA (Fig. 13 B).

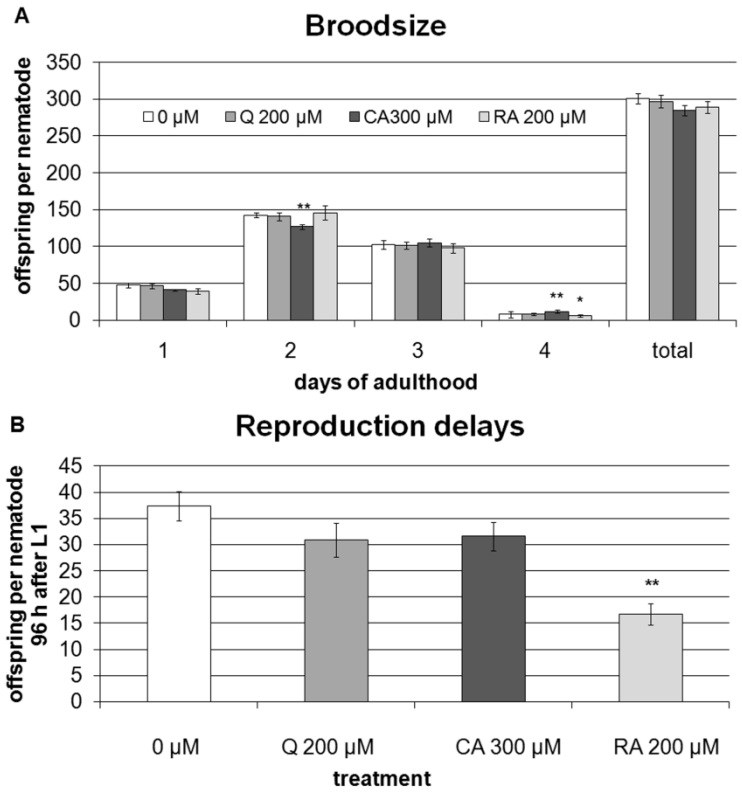



**Fig. 13 Reproduction. (A)** Displays the mean total broodsize per nematode for control compared to 200 µM Q, 300 µM CA and 200 µM RA treated animals. Three trials are compiled (n = 27/25/26). **(B)** PP treatment delays the onset of reproduction, results are significant for RA. Tests are conducted with wild type nematodes. Shown are two combined trials with n = 20 for each treatment; Bars represent the SEM. *p < 0.05, **p < 0.005 (One Way Anova).

### 3.4.2 PPs influence the growth of young larvae and adults

To test whether PPs influence the growth of wild type *C. elegans* the body size was measured using two different approaches: (i) the dynamics of growth were assessed by measuring the area of synchronized alive nematodes from 12 h until 180 h (~ 5$^{th}$ day of adulthood) after hatching with Image Pro Express software and (ii) the size of adults on their 6$^{th}$ day of adulthood was additionally quantified by measuring the length of heat-killed nematodes under a graduated eye pieced microscope. Both test designs demonstrate effects of PPs on nematode growth.

In general, exposure to PPs increased the volumetric area of living wild type larvae (Fig. 14 A), an effect that was lost by the time the worms reached the L4/young adult stage (60 h). This indicates that PPs may accelerate growth in early larval stages. Although this observation was deemed to be statistically robust, stage-specific differences in the magnitude of error bars may account for some, if not all, observed differences and calls for caution when interpreting the data set.

Figure 14 B reveals that all three PPs diminished the length of 6 days old dead wild type animals, although only the effects of CA and RA reached statistical significance. The mean length of the control was 1309.30 µM, Q treated nematodes sized averagely 1286.94 µM (98%), whereas CA treated worms measured 1267.20 µM (97%) and RA treated animals ranged around 1216.55 µM (93%). Overall these findings demonstrate that PPs do impact the growth of *C. elegans* although to varying extent, dependent on the developmental stage of the nematodes.

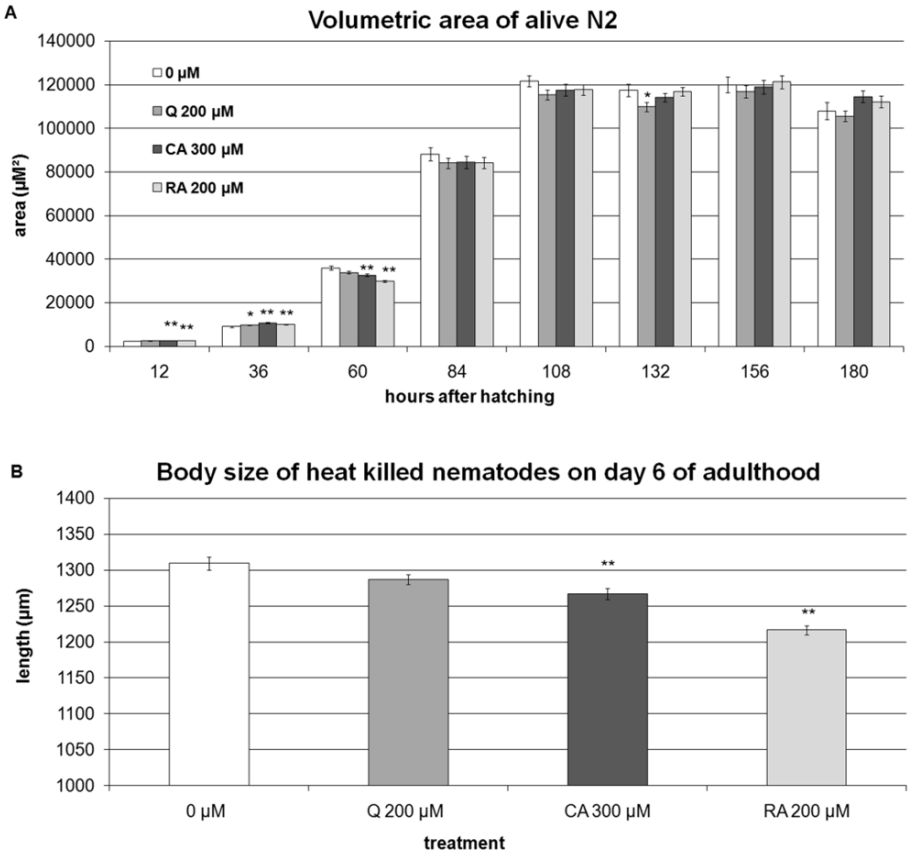



**Fig. 14 Growth. (A)** Impact of PPs on volumetric growth of living nematodes from 12 h after hatching until an advanced adult stage (180 h, 5th day of adulthood). Every 24 h worms were pictured and measured with Image Pro Express software. Pillars include two single trials, total number of n ranges between 22 and 79 for each treatment and day; **(B)** shows the length of heat-killed nematodes on their 6th day of adulthood. Bars compile 2 trials for CA (with total numbers n control/treated: 79/67) and 3 individual repeats for RA (n = 127/130). Tests are conducted with wild type worms. Error bars represent the SEM. *p < 0.05, **p < 0.005 (One Way Anova).

### 3.4.3 PPs change intestinal fat deposition

Since reduced intestinal fat deposition has been linked to longevity, the fat status of nematodes was determined. Taking into account the area and the sum of fluorescence per nematode (measured from lips to vulva), obvious differences in Nile Red staining were observed (Fig. 15 A). Q and CA exposed nematodes displayed reduced Nile Red fluorescence at days 3 and 6 of adulthood, a result that was significant for CA treated nematodes at day 3 and for Q treated worms at day 6 (representative images in Fig. 15 B and C, respectively). RA exposed *C. elegans* caused the mean fluorescence to increase marginally, however this result was not significant. Noteworthy, Nile Red fluorescence could not be reliably measured in young adult nematodes and 10 days old adults: in young adults the eggs seemingly are stained by Nile Red, thus excluding quantification of intestinal levels. In 10 days old adults the intestine structure obviously looses integrity in a large percentage of nematodes, which leads to misleading overstaining (representative images are displayed in Supplementary Fig. 1).

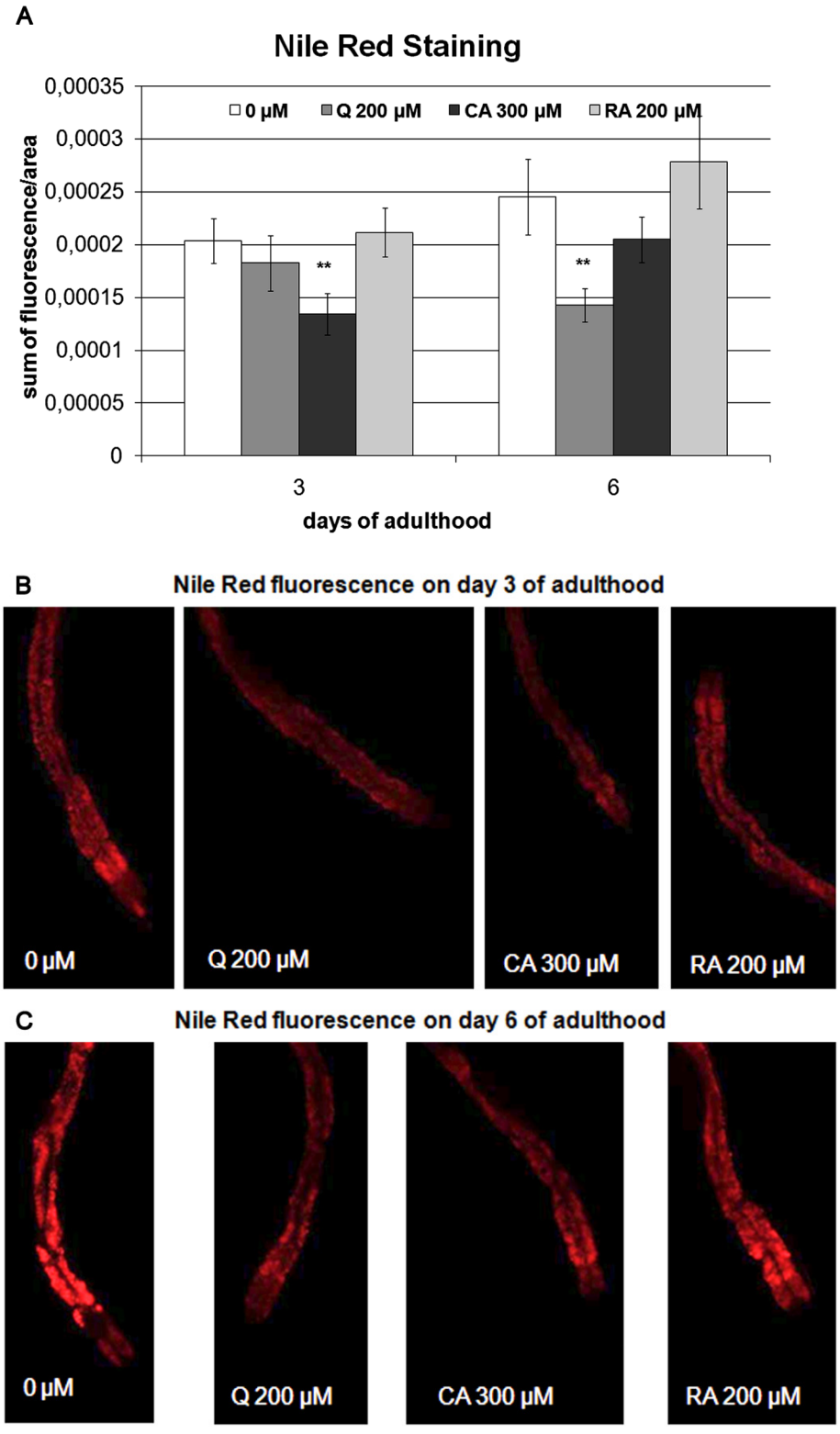



**Fig. 15 Fat deposition.** Nile Red staining of control and treated nematodes to detect possible PP-induced variations in fat storage on the 3rd and 6th day of adulthood. **(A)** The histogram represents the sum of fluorescence per area of nematode. Shown are two combined trials with a total of between 25 and 28 worms per treatment and day. Wild type *C. elegans* were used for. Bars represent the SEM. *$p < 0.05$ and **$p < 0.005$ (One Way Anova). **(B)** Displays respresentative fluorescence images of nematodes on day 3 of adulthood, **(C)** on day 6.

## 3.5 Antioxidative Properties

### 3.5.1 PP-distinct total antioxidative capacity (TAC) *in vivo* and *in vitro*

To prevent free radical damage, living organisms possess a defense system, to a major extent displayed by enzymatic (e.g. superoxide dismutases, glutathione peroxidases and catalase) and nonenzymatic antioxidants (e.g. ascorbic acid, tocopherol, urinic acid, billirubin). The cellular state of oxidative stress results from the imbalance between the formation of ROS and the ability to detoxify oxidizing radicals (Valko et al., 2007) and plays an important role in pathological processes and aging (Droge, 2002). Exogenous supplied compounds, which possess *in vivo* antioxidant properties can interfere in this process. To check if Q, CA and RA have the potential to act as antioxidants and thereby cause the life extension, their *in vitro* and *in vivo* antioxidative potential have been determined.

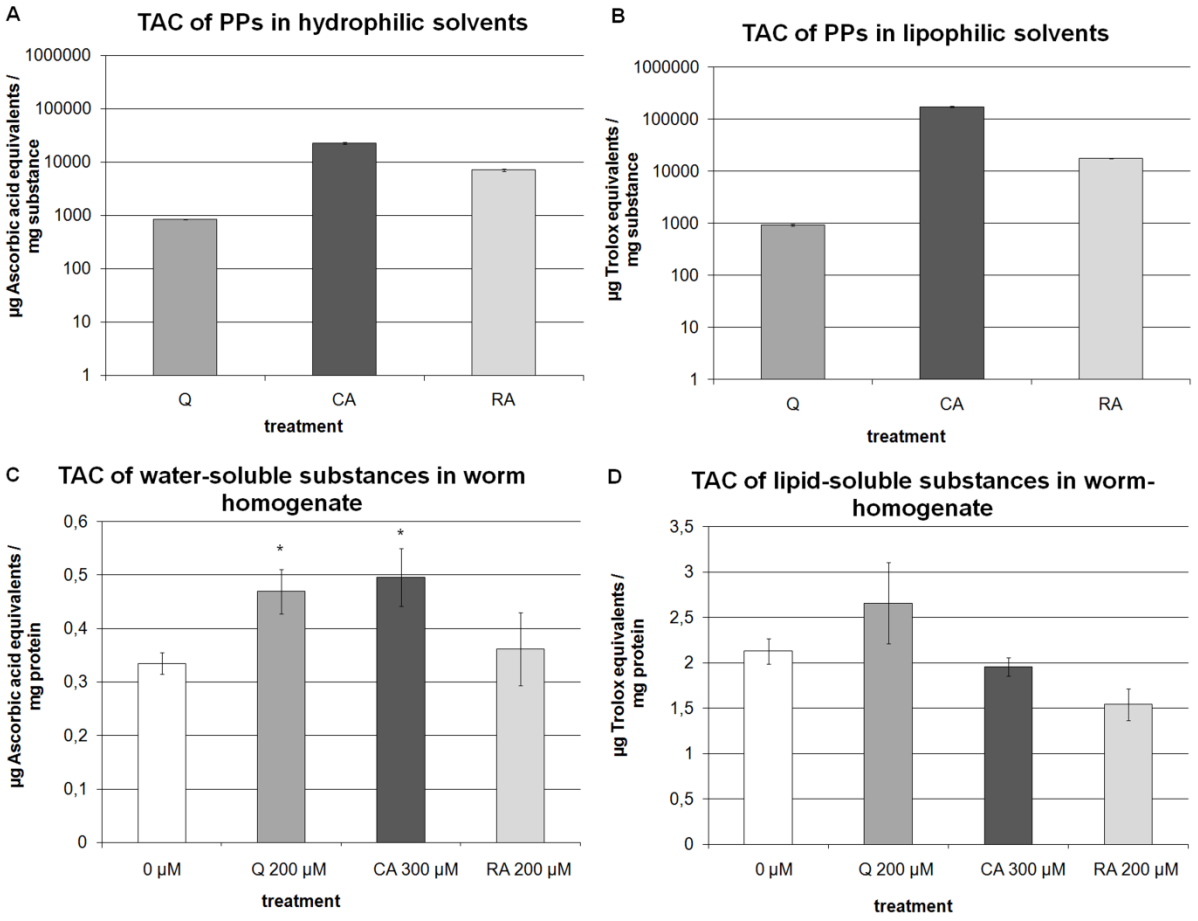



**Fig. 16 Total antioxidative capacity (TAC)** of PPs in hydrophilic **(A)** and lipophilic **(B)** solvents. TACs of water-soluble **(C)** or lipid-soluble **(D)** substances in homogenates obtained from PP treated and untreated wild type worms. Tests were repeated 3 times with a total of approximately 3000 worms per treatment in each single trial. Errors represent the SEM, differences were considered significant at *p < 0.05 (One Way Anova).

The antioxidative properties of pure PPs dissolved in either a hydrophilic solvent (water) or a lipophilic solvent (methanol) were measured and normalized to Ascorbic acid (AA) or Trolox (T), respectively. Although all three PPs are poorly soluble in water, the hydrophilic (Fig. 16 A) and the lipophilic (Fig. 16 B) extracts showed strong antioxidative properties. In both solvents PPs exhibited the same pattern relative to each other: the highest TAC value was observed with CA

(which was 22 and 175 fold higher than AA and T, respectively), followed by RA (7 fold higher than AA and 18 fold higher than T) and finally Q (0.8 fold of AA, 0.9 fold of T). To confirm the antioxidative activity *in vivo*, we measured the TAC in the homogenate of control, Q, CA and RA treated nematodes. The water-soluble fraction (Fig. 16 C) revealed the presence of significantly enhanced activities in case of Q and CA exposure, but not for RA treated worms. The TACs of lipid-soluble fractions (Fig. 16 D) were elevated by Q, suppressed by RA and similar to controls in CA exposures; none of the PP effects reached statistical significance.

### 3.5.2 Q prevents, RA enhances paraquat-induced matricidal

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) dichloride – a herbicide commonly used to generate oxidative stress *in vivo* by redox cycling - is metabolically reduced to the stable paraquat radical (PQ+) in a NAD(P)H-dependent reaction catalyzed by NAD(P)H-cytochrome P-450 reductase. In turn the paraquat radical reduces molecular oxygen and thereby produces the superoxide radical ($O_2^-$) (Brigelius et al., 1986; Fukushima et al., 1993; Yamada et al., 1993). The exact mechanism by which paraquat acts in detail is yet not enlightened. Presumably several components may contribute to the cytotoxicity of paraquat. However, strong evidence points towards ROS as main cause for the cytotoxicity of paraquat, which inflicts cellular injury by damaging biological macromolecules (Bus et al., 1976; Bagley et al., 1986; Chan and Weiss, 1987). This theory is supported by studies in diverse organisms, including bacteria (Greenberg et al., 1990; *Hongo et al., 1994*), *Drosophila* (Orr and Sohal, 1993; Parkes et al., 1993) and *C. elegans* (Vanfleteren, 1993), where elevated antioxidant enzyme activity in response to paraquat have been demonstrated.

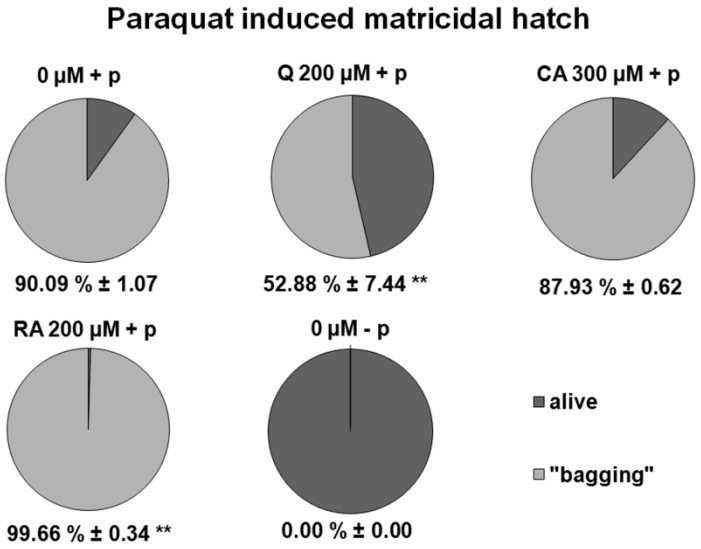



**Fig. 17 Prevention of paraquat-induced matricidal.** Displayed are the survival of wild type *C. elegans* 72 h after starting the treatment with 10 mM paraquat (+p) or a negative control (-p) combined with either 0 µM, 200 µM Q, 300 µM CA or 200 µM RA. Shown are data from two single trials. Total numbers of animals tested: control 283, Q 273, CA 285, RA 223, negative control 167. Numbers represent the percentage of observed "bagging" phenotype ± SEM; **p < 0.005 (Chi Square test).

Exposing control and PP treated nematodes for 72 h to paraquat caused severe levels of matricidal death ("bagging") (Fig. 17). In the absence of paraquat no "bagging" was observed throughout the whole time period. Nonetheless, a high degree of protection was provided by Q which was able to significantly increase the survival rate from 9.9 % (in control worms) to

47.1 %. Whilst increased survival by CA was statistically insignificant (to 12.1 %), RA imposed a negative effect, reducing the survival to 0.3 %. In conclusion, Q was shown to rescue a paraquat induced “bagging” phenotype suggesting that Q may play an effective role in protecting against oxidative stress.

So far, induction of matricidal hatching in *C. elegans* have been observed following starvation, due to placement in water without food, or caused by various stress conditions, as when exposed to chitinase-producing bacteria or high salt concentrations (Chen and Caswell-Chen, 2003). It is arguable, if the observed internal hatching observed in this study arises partly due to the inhibitory impact of paraquat on the bacterial growth (personal communication with Zeitoun), which may alter the food conditions for the nematodes towards a caloric restrictive diet, known to cause matricidal. But beside, paraquat doubtlessly acts in a direct oxidative way on the worms.

### 3.5.3 All three PPs prolong the lifepan of *mev-1* mutants

The first mitochondrial mutation identified in *C. elegans* was *mev-1(kn1).* Ishii et al. (1998) characterized *mev-1* as a missense mutation in the cytochrome b subunit of complex II. Electron chain transport studies with mitochondria from *mev-1* nematodes (Senoo-Matsuda et al., 2001; Kayser et al., 2004) showed a decreased complex II activity and also decreased rates of oxidative phosphorylation. *mev-1* has been characterized to be sensitive to oxygen, to show increased rates of oxidative damaged proteins, a diminished fecundity (Brand, 2000), an elevated production of superoxide (Senoo-Matsuda et al., 2001) and thereby a shortened lifespan, which can be extended to normal by add-on of superoxide dismutase/catalase mimetics (Sedensky and Morgan, 2006). Kondo et al. (2005) also reported that DAF-16 - a fork-head transcription factor which is negatively regulated by the ILS pathway - persists constitutively in the nuclei of *mev-1* nematodes even under normal growth conditions, in contrast to the common situation in wild type animals, where DAF-16 remains in the cytoplasm until a translocation is triggered by diverse activating stimuli (e.g. environmental stressors, such as starvation, heat and oxidative stress; Henderson and Johnson, 2001). Kondo et al. (2005) assumed that the endogenously created high steady-state levels of oxidative stress caused the persistence of nuclear DAF-16 in *mev-1* animals and overwhelm all resistance mechanisms mediated by the translocated transcription factor. A reversal of the nuclear DAF-16 state could be achieved by supplementation of the antioxidant, radical scavenger coenzyme Q10 (Kondo 2005). All these findings point toward the heightened ROS production as origin of the diminished lifespan found in *mev-1* nematodes, rather than the inhibited oxidative phosphorylation levels.

Since Q, CA and RA were able to extend the mean survival time of *mev-1* mutants by a highly significant 10 %, 10 % and 8% (Fig. 18 A, B and C, for more details see also Tables 6, 8, 9 in sections 3.6.1 and 3.6.2) suggests that all three PPs are able to enhance survival during oxidative stress predominating in *mev-1* mutants.

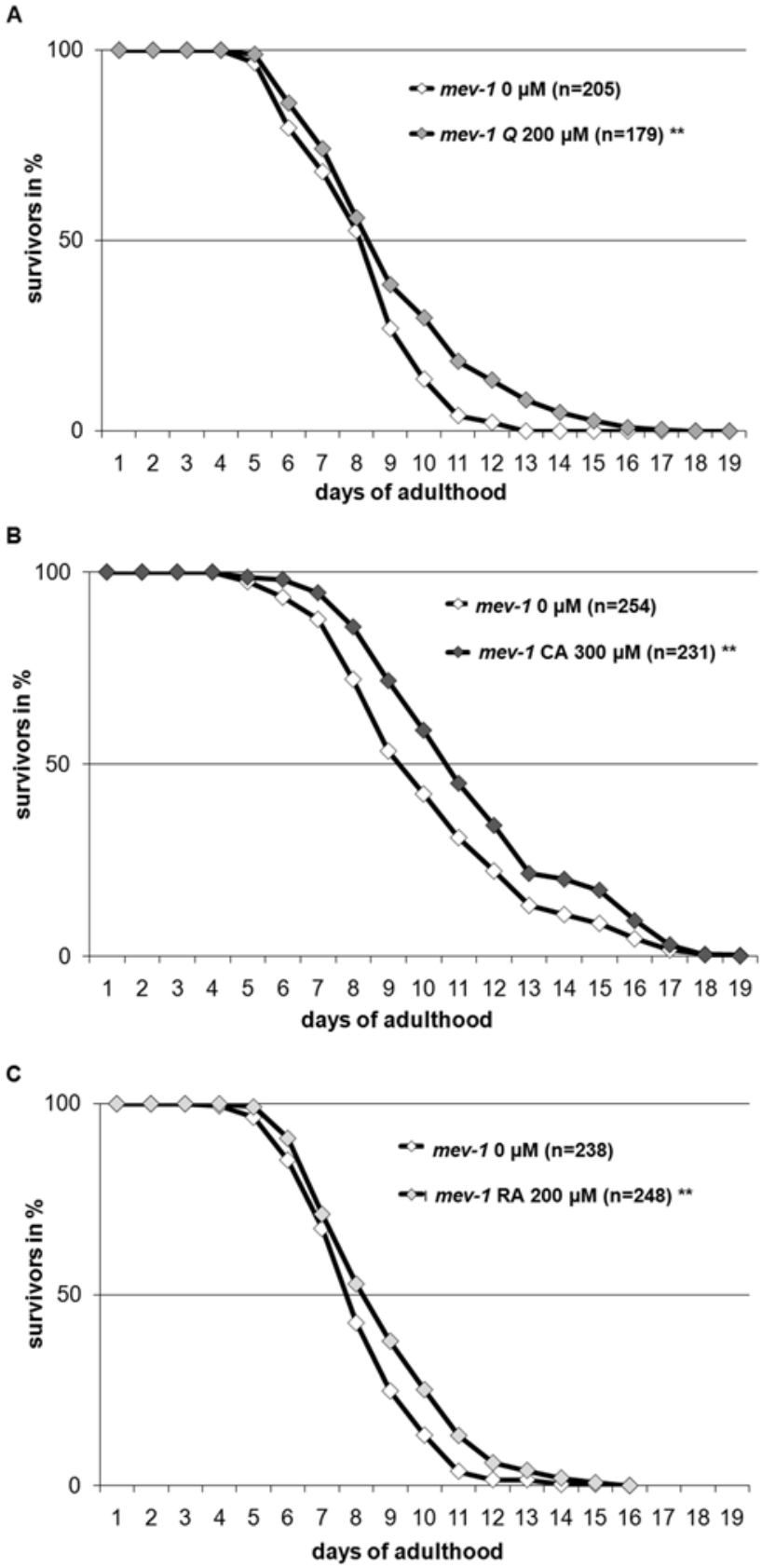



**Fig. 18 Lifespan of *mev-1* mutants treated with 200 µM Q (A), 300 µM CA (B) or 200 µM RA (C).** Appropriate data (mean and median lifespan, n and trial numbers, as well as single trial significances) are listed in Tables 6, 8 and 9; differences were considered significant at **p < 0.005 (log-rank test).

### 3.5.4 Intestinal lipofuscin fluorescence is decreased due to Q and CA

One of the markers for oxidative damage during aging is the intensity of lipofuscin, which accumulates gradually in the secondary lysosomes of aging organisms (Brunk and Terman, 2002) including *C. elegans* (Hosokawa et al., 1994). Lipofuscin consists primarily of lipid peroxidation products and oxidized proteins that resist proteolytic degradation (Berdichevsky et al., 2010). The quantification of lipofuscin fluorescence intensity (Fig. 19 A) demonstrates that Q, CA and RA exposure was able to reduce lipofuscin accumulation by a significant 16% and 34% and a nonsignificant 7%, respectively. Images in Fig. 19 B show representative images of treated and control nematodes.

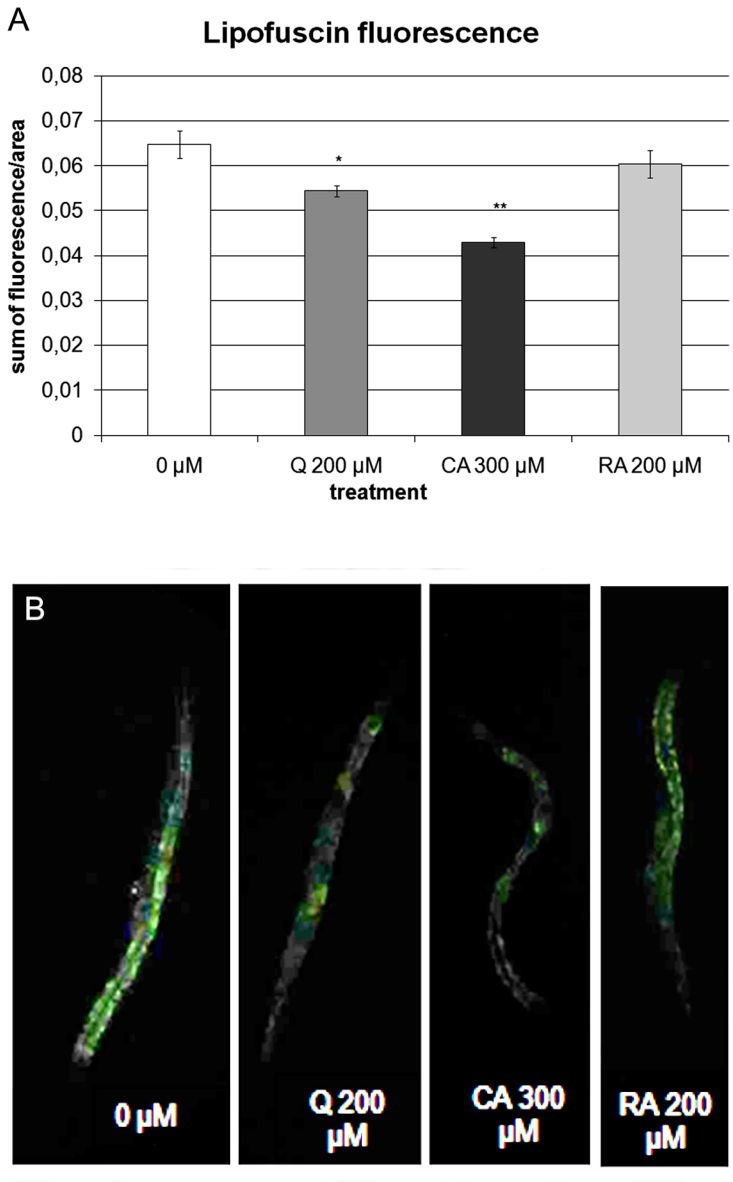



**Fig. 19 Intestinal Lipofuscin fluorescence in wild type nematodes at the 7th day of adulthood. (A)** Graphs of combined results of about 40 worms per treatment. Errors represent the SEM, differences were considered significant at *p < 0.05 (One Way Anova). **(B)** Representative lipofuscin fluorescence pictures.

## 3.6 Genetic background: mutant lifespan and thermotolerance assays

To unravel the underlying genetics of Q, CA and RA action *in vivo,* lifespan assays were carried out with selected *C. elegans* mutant strains lacking genes previously implicated in stress response or aging. Due to Q being the main focus of this study, 13 mutant strains are investigated with Q, whereas in CA and RA 8 mutant strains were used (detailes about mutated genes can be found in the discussion section 4.6.2, Table 25). Specifically, lifespan assays with CA and RA focused on mutants including the ones found to affect Q (Pietsch et al., 2009) and blueberry PP (Wilson et al., 2006) mediated longevity.

### 3.6.1 Genetic players of Q mediated longevity

**Genes required for Q mediated lifespan extension**

Fig. 19 and Table 5 summarize results from mutant lifespan assays. It has been shown that the polyphenol resveratrol can increase lifespan via the sirtuin gene *sir-2.1*, which encodes a histone deacetylase-like protein that integrates the metabolic status and lifespan (Tissenbaum and Guarente, 2001; Wood et al., 2004). Q treatment extended lifespan of *sir-2.1(ok434)*, suggesting that Q does not modulate this pathway. Several *C. elegans* transcription factors, including DAF-16, NHR-8 and SKN-1, promote the expression of antioxidant or detoxification enzymes. Q treatment was able to prolong lifespan of *daf- 16(mgDf50)*, *nhr-8(ok186)* and *skn-1(zu67)* nematodes, indicating that Q may act independently of these genes as well. A similar result was obtained for *jnk-1(gk7)*. Activation of JNK-1, which is localized in the neuronal system, drives the nuclear translocation of DAF-16 within peripheral cells (Wolf et al., 2008). Furthermore, the lifespan of *daf-12(m20)* (daf-defective (daf-d) allel: mutants lack the ability to form dauers) could also be enhanced by Q. DAF-12, a member of the steroid hormone receptor superfamily, influences dauer formation downstream of the TGF-beta and ILS pathways, and affects gonad-dependent adult longevity together with DAF-16 (Gerisch et al., 2007). In contrast to the aforementioned mutants, Q treatment did not prolong the lifespan of two long-lived and oxidative stress-resistant mutants, namely *daf-2(e1368)* and *age-1(hx546)*. Both genes are key players of the ILS pathway, and mutations result in a dramatic increase in longevity. DAF-2 is the only insulin/IGF-1 like receptor in *C. elegans*, AGE-1 a catalytic subunit of a phosphoinositide 3-kinase (PI3K), is downstream of DAF-2. Both enzymes act at a similar point in the genetic epistasis pathway for dauer arrest and longevity and negatively regulate the activity of DAF-16. Given that DAF-16 was found to be non essential for lifespan regulation by Q, it is perhaps surprising to find other members of the ILS family to be involved in Q-mediated longevity. This issue will be addressed in finer detail concerning the DNA-microarray results.

Furthermore, a disrupted calmodulin kinase II (CaMKII) pathway seems to prevent Q induced longevity. Survival in hyperosmotic environments requires the activity of several proteins of the CaMKII pathway, in which OSR-1 is coupled to SEK-1 (MAPKK) through UNC-43 (CaMKII) (Solomon et al., 2004). SEK-1 is also required for the resistance to pathogenic bacteria and oxidative stress by assisting the translocation of cytoplasmic DAF-16 into the nucleus (Kim et al., 2002; Kondo et al., 2005). All three genes were found to be essential for blueberry extract-mediated longevity (Wilson et al., 2006). Similarly, Q treatment was not able to prolong the lifespan of *unc-43(n498n1186)* and *sek-1(ag1)* mutants, strongly suggesting that this flavonoid may act via this pathway. Noteworthy however, was that *osr-1(rm1)* nematodes continued to display robust Q induced longevity (Fig. 20, Table 6)

**Table 6 Lifespan data of Q 200 µM treated mutant nematodes.** Effect of Q 200 µM treatment on mutant *C. elegans* mean lifespan compared to untreated control. Genetic backgrounds preventing significant Q effects on *C. elegans*' lifespan are highlighted in grey (refers to Fig. 20)

| Genotype | Median lifespan ± SEM (d) | | Change | Mean lifespan ± SEM (d) | | Change | n Control/Treated | Trials | Change mean lifespan single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control (d) | Treated (d) | | Control (d) | Treated (d) | | | | |
| N2 | 16.09±0.72 | 17.64±0.77 | 1.10±0.04 | 16.43±0.77 | 18.05±0.84 | 1.10$^{*}$±0.03 | 806/865 | 7 | 1.07$^{*}$, 1.25$^{**}$, 1.04, 1.07, 1.12$^{**}$, 1.03, 1.06$^{*}$ |
| N2[a] | 19.24±0.28 | 20.75±0.26 | 1.09±0.01 | 19.73±0.32 | 21.48±0.54 | 1.09$^{*}$±0.02 | 146/122 | 2 | 1.11$^{**}$, 1.07$^{*}$ |
| *age-1(hx546)* | 17.76±0.87 | 18.53±0.78 | 1.04±0.09 | 20.19±0.81 | 20.50±0.53 | 1.02±0.04 | 643/599 | 5 | 0.93, 1.01, 1.06, 1.09, 0.98 |
| *akt-2(ok393).* | 17.54±1.40 | 19.62±1.93 | 1.12±0.02 | 18.42±0.95 | 20.21±0.82 | 1.10$^{**}$±0.02 | 284/241 | 2 | 1.08$^{*}$, 1.11$^{**}$ |
| *daf-2(e1368)* | 25.06±1.29 | 24.93±1.22 | 0.99±0.05 | 25.08±0.92 | 25.57±0.84 | 1.00±0.02 | 378/353 | 4 | 0.94, 1.01, 1.05, 0.97 |
| *daf-12(m20)* | 10.26±0.64 | 12.67±1.22 | 1.23±0.04 | 11.53±0.36 | 13.35±0.86 | 1.16$^{**}$±0.05 | 207/214 | 2 | 1.11$^{*}$, 1.20$^{**}$ |
| *daf-16(mgDf50)* | 10.30±0.87 | 12.30±1.13 | 1.19±0.02 | 11.07±0.74 | 12.72±0.85 | 1.15$^{**}$±0.00 | 475/512 | 3 | 1.15$^{**}$, 1.15$^{**}$, 1.14$^{**}$ |
| *jnk-1(gk7)* | 12.94±1.57 | 14.75±2.40 | 1.14±0.05 | 13.82±0.87 | 15.26±1.10 | 1.11$^{**}$±0.01 | 305/307 | 2 | 1.09$^{**}$, 1.11$^{**}$ |
| *mev-1(kn1)* | 7.74±0.84 | 8.09±0.56 | 1.05±0.04 | 8.38±0.46 | 9.25±0.15 | 1.10$^{**}$±0.06 | 205/179 | 2 | 1.16$^{**}$, 1.05 |
| *nhr-8(ok186)* | 13.36±0.11 | 16.11±0.69 | 1.21±0.04 | 14.73±0.19 | 16.49±0.22 | 1.12$^{**}$±0.04 | 324/344 | 2 | 1.08$^{**}$, 1.16$^{**}$ |
| *osr-1(rm1)* | 14.64±2.87 | 17.82±3.96 | 1.22±0.04 | 15.90±1.76 | 17.74±1.84 | 1.12$^{**}$±0.01 | 156/223 | 2 | 1.13$^{*}$, 1.11$^{*}$ |
| *sek-1(ag1)*[a] | 16.34±0.16 | 16.23±1.49 | 0.99±0.08 | 16.77±0.14 | 17.13±0.99 | 1.02±0.05 | 133/101 | 2 | 1.05, 0.97 |
| *sir-2.1(ok434)* | 14.25±1.24 | 15.54±1.12 | 1.09±0.03 | 14.72±0.57 | 15.92±0.64 | 1.08$^{**}$±0.03 | 262/261 | 3 | 1.07, 1.05, 1.13$^{**}$ |
| *skn-1(zu67)* | 10.25±0.94 | 11.95±2.46 | 1.17±0.09 | 12.01±0.64 | 14.19±1.11 | 1.18$^{**}$±0.04 | 262/283 | 2 | 1.14$^{**}$, 1.21$^{**}$ |
| *unc-43 (n498n1186)* | 10.13±2.39 | 10.14±2.14 | 1.00±0.01 | 11.50±1.60 | 11.27±1.35 | 0.98±0.02 | 283/293 | 2 | 1.01, 0.96 |

* Statistical significance was calculated by log-rank test, changes in mean lifespan considered significant at *p < 0.05 and **p < 0.005

[a] incubated at 15°C

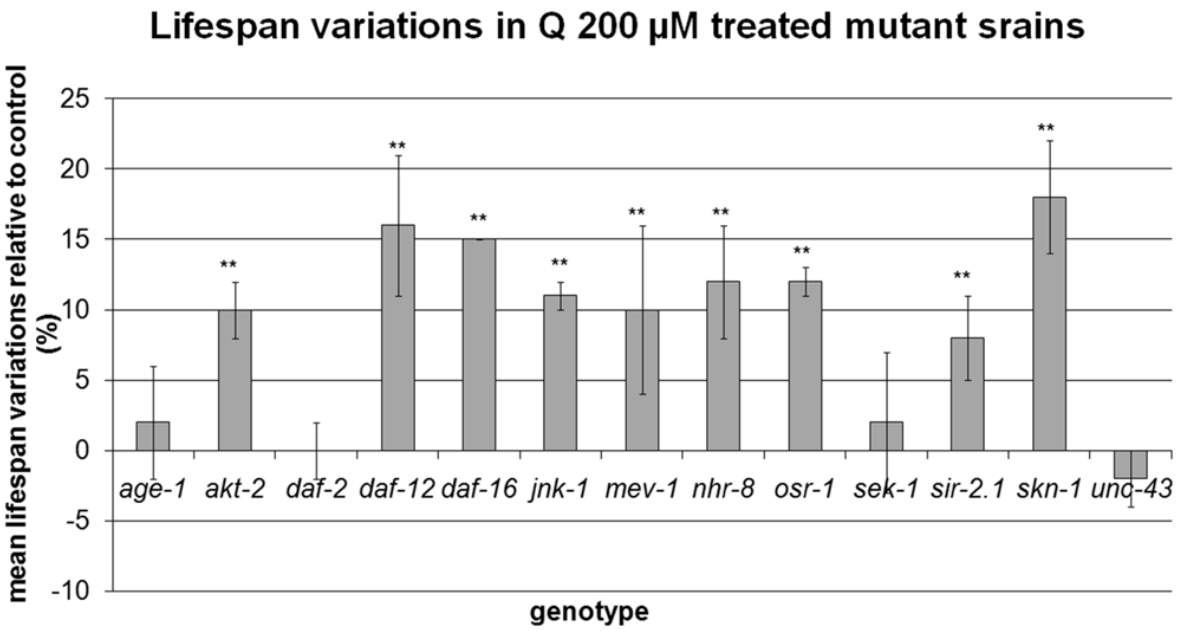



**Fig. 20 Genetic modulators of Q mediated longevity.** Lifespan alterations in 13 mutant strains following the treatment with 200 µM Q. Shown are the mean lifespan variations compared to the respective untreated control. Mean and median lifespans, n and trial numbers, single trial significances are listed in Table 6. Error bars represent SEM, **p < 0.005 (log-rank test).

**Are the genes required for Q mediated lifespan extension also responsible for enhanced thermotolerance?**

Longevity is often coupled to stress resistance in *C. elegans* (Johnson et al., 2002), therefore the presumable genetic players for Q mediated lifespan extension were reassessed by thermotolerance assays (Fig. 21, Table 7). The thermal resistance was significantly increased in wild type (N2) animals treated with 200 µM Q (according to Saul et al., 2008), a trend that could not be observed in *age-1(hx546)*, *daf-2(e1368)*, *sek-1(ag1)* and *unc-43(n498n1186)* mutants. These findings support the lifespan assays and strengthen the notion that Q mediated longevity is driven by *age-1*, *daf-2*, *sek-1* and *unc-43*.

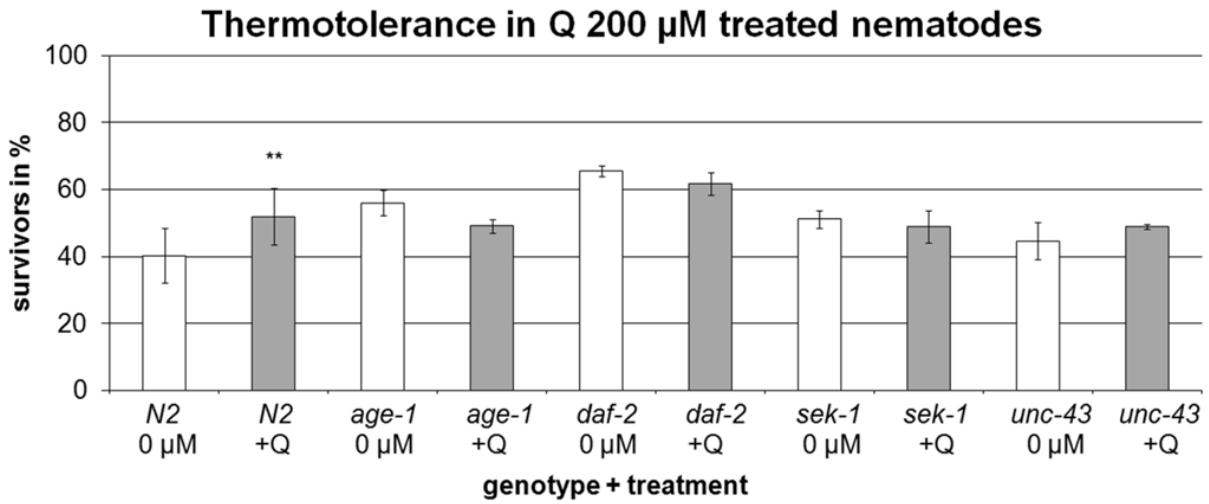



**Fig. 21 Thermal stress resistance of wild type (N2) and selected mutant strains untreated (0 µM) or treated with Q 200 µM.** Shown are the 8 h-survival rates for N2 and *unc-43(n498n1186)*, 9 h-survival rates for *age-1(hx546)*, 10 h survival rates for *daf-2(e1368)*, and the 6 h-survival rates for *sek-1(ag1)* exposed to 35°C. The experiment was conducted with 6th days old adult. Mean values, n, trial numbers, single trial results and significances are listed in Table 7. **p < 0.005 (Chi Square test).

**Table 7 Thermal resistance of Q 200 µM treated nematodes.** Effect of 200 µM Q on thermal resistance of N2 and four different mutant strains compared to respective untreated control (refers to Fig. 21).

| Genotype | Hours of heat stress | Survivors (%) all trials pooled ± SEM | | n Control/ Treated | Trials | Survivors (%) single trials ± SEM | | n Control / Treated |
|---|---|---|---|---|---|---|---|---|
| | | Control (0 µM) | Treated (Q 200 µM) | | | Control (0 µM) | Treated (Q 200 µM) | |
| N2 | 8 h | 40.31±8.12 | 51.95±8.53** | 251/273 | 3 | 45.52±7.64 | 56.19±0.84 | 120/144 |
| | | | | | | 24.39±8.84 | 35.53±5.55 | 82/76 |
| | | | | | | 51.02±5.85 | 64.15±9.16 | 49/53 |
| *age-1 (hx546)* | 9 h | 55.91±3.78 | 49.09±2.14 | 303/337 | 3 | 62.07±3.92 | 53.23±5.37 | 116/124 |
| | | | | | | 49.04±7.16 | 46.09±2.34 | 104/115 |
| | | | | | | 56.63±2.13 | 47.96±6.53 | 83/98 |
| *daf-2 (e1368)* | 10 h | 65.42±1.58 | 61.80±3.33 | 305/309 | 3 | 68.47±3.41 | 66.47±4.06 | 111/117 |
| | | | | | | 64.65±7.47 | 63.30±9.02 | 99/109 |
| | | | | | | 63.16±5.80 | 55.42±6.90 | 95/83 |
| *sek-1 (ag1)* | 6 h | 51.14±2.63 | 48.78±4.82 | 98/111 | 3 | 46.15±0.00 | 52.63±0.23 | 26/38 |
| | | | | | | 52.17±1.57 | 54.55±4.08 | 23/22 |
| | | | | | | 55.10±8.94 | 39.22±8.57 | 49/51 |
| *unc-43 (n498n1186)* | 8 h | 44.67±5.44 | 48.86±0.79 | 299/341 | 3 | 55.00±7.31 | 47.71±1.98 | 100/109 |
| | | | | | | 36.56±7.16 | 48.48±4.94 | 93/99 |
| | | | | | | 42.45±5.06 | 50.38±1.94 | 106/133 |

** p < 0.005

### 3.6.2 Genetic players of CA and RA mediated longevity

**Genes required for CA and RA mediated lifespan extension**

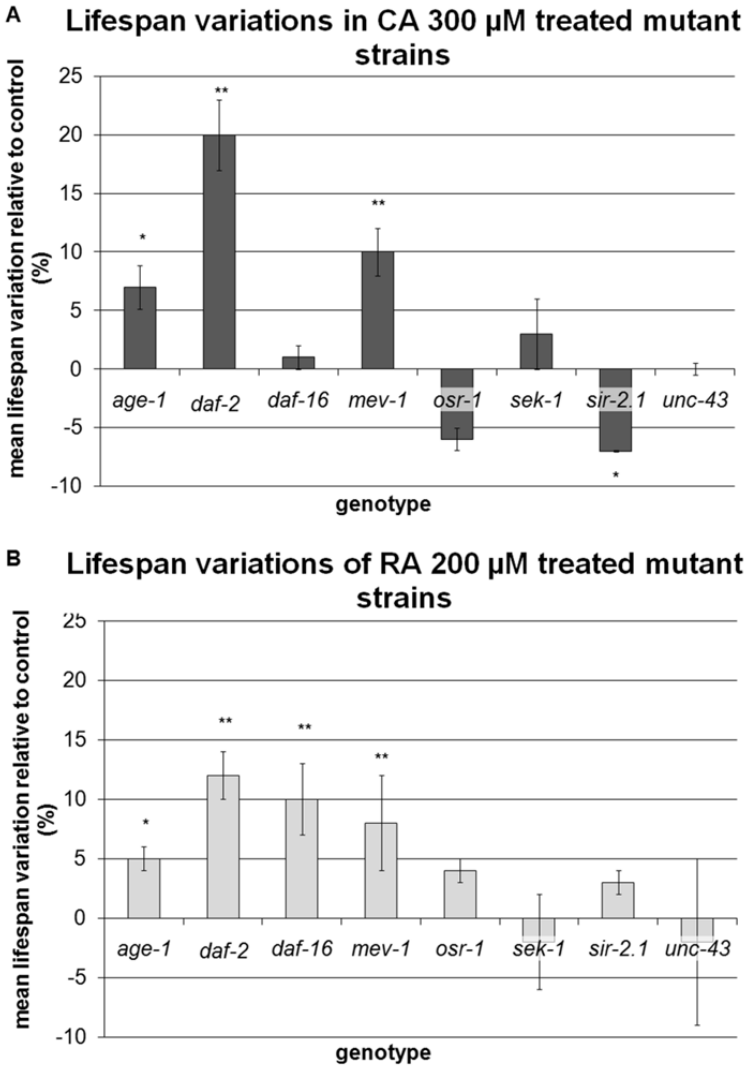



**Fig. 22 Genetic modulators of CA (A) or RA (B) mediated longevity.** Lifespan alterations in eight mutant strains following the treatment with either 300 µM CA or 200 µM RA. Shown are the mean lifespan variations compared to the respective untreated control. Mean and median lifespans, n and trial numbers, single trial significances are listed in Tables 8 (CA) and 9 (RA). Error bars represent SEM, *p < 0.05; **p < 0.005 (log-rank test).

Since Q mediated longevity was specifically abolished in *age-1*, *daf-2*, *sek-1* and *unc-43* mutants, the effects of CA and RA were analyzed in these as well as other mutants. CA and RA could not significantly extend lifespan in the four mutant strains (*osr-1*, *sek-1*, *sir-2.1* and *unc-43*) (Fig 22 A and B, Tables 8 and 9) This suggests that CA and RA both use comparable genetic pathways for life extension which are, however, in parts distinct from those involved in Q action. Notably, lifespan was even reduced in CA exposed *sir-2.1* and *osr-1* and RA treated *sek-1* and *unc-43*, however, it should be mentioned, that the individual trials of *unc-43* resulted in highly variable, indeed sometimes contradictory results (changes: 0.85$^{**}$, 1.00 and 1.10$^{**}$). One difference prevailed throughout; in *daf-16* lifespan extension was only achieved by RA and not CA.

**Table 8 Lifespan data of CA 300 µM treated mutant nematodes.** Effect of CA 300 µM treatment on mutant *C. elegans* mean lifespan compared to untreated control. Genetic backgrounds preventing significant CA effects on *C. elegans*' lifespan are highlighted in grey (refers to Fig. 22 A).

| Genotype | Median lifespan (d) ± SEM | | Change ± SEM | Mean lifespan (d) ± SEM | | Change ± SEM | n Control/ Treated | Trials | Mean lifspan change single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Treated | | Control | Treated | | | | |
| *age-1 (hx546)* | 17.30±1.96 | 19.29±2.31 | 1.11±1.29 | 19.09±1.13 | 20.40±1.56 | 1.07*±1.85 | 270/279 | 3 | 1.10, 1.05, 1.11 |
| *daf-2 (e1368)* | 19.22±3.42 | 21.64±2.89 | 1.13±0.05 | 20.20±2.61 | 24.24±2.46 | 1.20**±0.03 | 144/127 | 2 | 1.22*, 1.16* |
| *daf-16 (mgDf50)* | 11.45±1.10 | 11.96±1.43 | 1.04±0.02 | 12.11±0.99 | 12.27±1.11 | 1.01±0.01 | 334/337 | 2 | 1.02, 1.00 |
| *mev-1 (kn1)* | 9.85±1.66 | 11.05±1.51 | 1.12±0.04 | 10.85±1.63 | 11.98±1.66 | 1.10**±0.02 | 254/231 | 2 | 1.10**, 1.14** |
| *osr-1 (rm1)* | 9.93±0.07 | 9.73±0.27 | 0.98±0.03 | 10.77±0.09 | 10.15±0.19 | 0.94±0.01 | 97/103 | 2 | 95.41, 93,53 |
| *sek-1 (ag1)* | 15.10±1.23 | 15.67±0.33 | 1.04±0.06 | 16.19±0.42 | 16.64±0.86 | 1.03±0.03 | 63/66 | 2 | 0.99, 1.05 |
| *sir-2.1 (ok434)* | 13.01±0.11 | 11.89±0.03 | 91.37±0.01 | 14.01±0.06 | 13.08±0.05 | 0.93*±0.00 | 130/144 | 2 | 0.94, 0.94 |
| *unc-43 (n498n1186)* | 15.77±1.45 | 15.60±1.37 | 0.99±0.00 | 15.84±1.30 | 15.91±1.37 | 0.99±0.01 | 321/298 | 2 | 0.99, 0.98 |

*p < 0.05
**p < 0.005

**Table 9 Lifespan data of RA 200 µM treated mutant nematodes.** Effect of RA 200 µM treatment on mutant *C. elegans* mean lifespan compared to untreated control. Genetic backgrounds preventing significant RA effects on *C. elegans*' lifespan are highlighted in grey (refers to Fig. 22 B).

| Genotype | Median lifespan (d) ± SEM | | Change ± SEM | Mean lifespan (d) ± SEM | | Change ± SEM | n Control/ Treated | Trials | Mean lifespan change single trials |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Treated | | Control | Treated | | | | |
| *age-1 (hx546)* | 16.63±1.26 | 18.24±1.61 | 1.10±0.04 | 19.20±0.95 | 20.14±0.90 | 1.05*±0.01 | 370/400 | 3 | 1.07*, 1.04, 1.04 |
| *daf-2 (e1368)* | 21.59±0.74 | 24.75±0.93 | 1.15±0.06 | 21.78±0.63 | 24.46±0.46 | 1.12**±0.02 | 174/203 | 3 | 1.12*, 1.16*, 1.08 |
| *daf-16 (mgDf50)* | 11.35±1.40 | 12.59±1.44 | 1.11±0.04 | 12.02±1.30 | 13.12±1.29 | 1.10**±0.03 | 709/771 | 3 | 1.09**, 1.15**, 1.05* |
| *mev-1 (kn1)* | 7.72±0.47 | 8.16±0.95 | 1.06±0.06 | 8.29±0.34 | 8.94±0.93 | 1.08**±0.04 | 238/148 | 3 | 1.11**, 1.04 |
| *osr-1 (rm1)* | 15.05±1.16 | 14.74±1.16 | 0.98±0.06 | 13.86±0.24 | 14.44±0.13 | 1.04±0.01 | 228/282 | 2 | 1.03, 1.05 |
| *sek-1 (ag1)* | 15.05±0.20 | 14.74±1.16 | 0.98±0.06 | 16.19±0.73 | 15.94±1.20 | 0.98±0.04 | 70/78 | 2 | 1.02, 0.95 |
| *sir-2.1 (ok434)* | 13.46±1.28 | 14.04±1.22 | 1.04±0.02 | 14.28±0.92 | 14.69±1.02 | 1.03±0.01 | 277/249 | 3 | 1.01, 1.05, 1.02 |
| *unc-43 (n498n1186)* | 12.05±1.76 | 11.68±1.75 | 0.97±0.09 | 12.38±1.35 | 12.06±1.25 | 0.98±0.07 | 521/538 | 3 | 0.85**, 1.00, 1.10** |

*p < 0.05
**p < 0.005

**Verification of genetic palyers for CA and RA by thermotolerance**

The thermal resistance is significantly enhanced in CA and RA treated nematodes (Fig. 23 A and B). To support findings of genetic players for longevity thermotolerance assays with mutant strains that displayed no life extension due to respective PP treatment were also conducted. The results from different trials were somewhat variable, and in some cases contradictory (for detailed summary of results see Tables 10 and 9), however some valuable observations could be extracted which reflect general trends rather than firm mechanistic evidence. Taking all findings together, strengthens the notion that *daf-16*, *sek-1*, *sir-2.1, unc-43* and tentatively *osr-1* modulate the health gaining action of CA (Fig. 23 A). Results for RA are illustrated in Fig. 23 B. Independent repeats revealed that treated and untreated *osr-1* showed almost equal numbers of survivors. The same holds true for *unc-43* and *sek-1*. Treated *sir-2.1* displayed in three independent experiments moderate but statistically insignificant increased thermotolerance, a result that is analogous to the lifespan assays. In summary, *osr-1*, *sek-1* and *unc-43* seem to be genetic mediators of RA *in vivo* responses, with *sir-2.1* possibly playing an ancillary role.

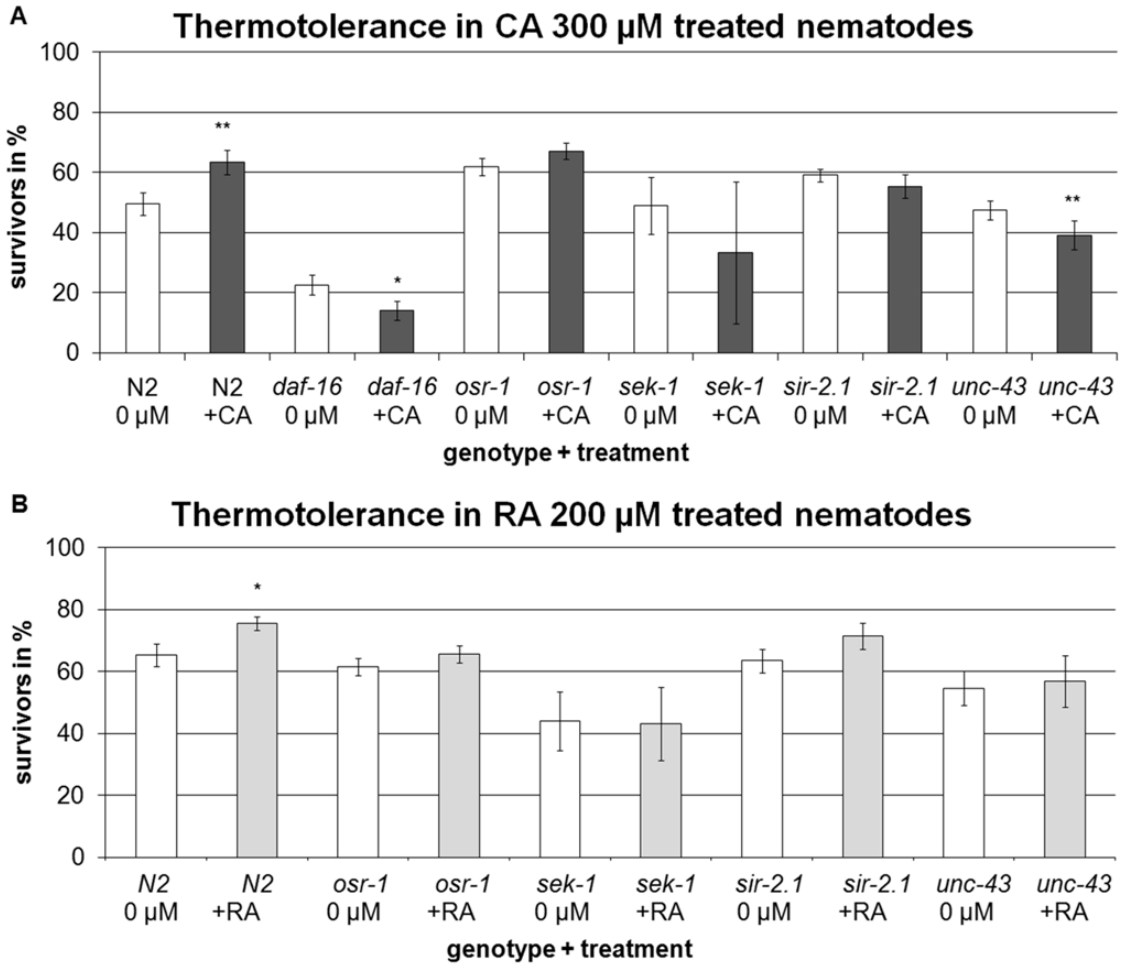



**Fig. 23 Thermal stress resistance of N2 and selected mutant strains** untreated (0 µM) or treated with **(A)** 300 µM CA or **(B)** 200 µM RA, respectively. Shown are the 8 h-survival rates for N2 and *unc-43(n498n1186)*, 7 h-survival rates for *sir-2.1(ok434)*, *osr-1(rm1)* and *daf-16(mgDf50)*, and the 6 h-survival rate for *sek-1(ag1)* exposed to 35°C. The experiment was conducted with 6$^{th}$ days old adult. Mean values, n, trial numbers, single trial results and significances are listed in Tables 10 (CA) and 11 (RA). *p < 0.05, **p < 0.005 (Chi Square test).

**Table 10 Thermal resistance of CA 300 µM treated nematodes.** Effect of 300 µM CA on thermal resistance of N2 and five different mutant strains compared to respective untreated control (refers to Fig. 23 A).

| Genotype | Hours of heat stress | Survivors (%) all trials pooled ± SEM | | n Control/ Treated | Trials | Survivors (%) single trials± SEM | | n Control / Treated |
|---|---|---|---|---|---|---|---|---|
| | | Control (0 µM) | Treated (CA 300 µM) | | | Control (0 µM) | Treated (CA 300 µM) | |
| N2 | 8 h | 49.34±3.85 | 62.55**±4.07 | 494/466 | 6 | 67.86±3.48 | 77.32±5.58 | 112/97 |
| | | | | | | 53.33±9.43 | 81.82*±5.51 | 30/33 |
| | | | | | | 69.05±2.99 | 74.16±4.87 | 84/89 |
| | | | | | | 58.33±4.82 | 75.44*±5.71 | 108/114 |
| | | | | | | 26.94±8.36 | 38.03*±7.48 | 193/142 |
| | | | | | | 45.87±3.36 | 57.85±4.95 | 109/121 |
| *daf-16 (mgDf50)* | 7 h | 22.54±3.31 | 14.07*±3.09 | 173/199 | 2 | 21.88±6.02 | 15.90±4.31 | 96/88 |
| | | | | | | 23.38±4.06 | 12.61±5.37 | 77/111 |
| *osr-1 (rm1)* | 7 h | 61.95±2.85 | 67.08±2.64 | 205/240 | 2 | 58.63±4.50 | 68.03±3.04 | 108/123 |
| | | | | | | 64.37±2.32 | 66.52±4.78 | 97/117 |
| *sek-1 (ag1)* | 6 h | 48.98±9.53 | 33.33±23.55 | 49/48 | 3 | 25.00±0.00 | 10.00±0.00 | 8/10 |
| | | | | | | 53.85±0.00 | 26.67±0.00 | 26/30 |
| | | | | | | 53.33±0.00 | 87.50±0.00 | 15/8 |
| *sir-2.1 (ok434)* | 7 h | 59.11±2.09 | 55.36±3.82 | 181/168 | 2 | 55.95±1.67 | 53.62±3.33 | 84/69 |
| | | | | | | 61.86±3.34 | 56.57±7.52 | 97/99 |
| *unc-43 (n498n1186)* | 8 h | 47.41±3.26 | 39.12**±4.86 | 580/611 | 6 | 46.94±7.71 | 44.58±5.89 | 98/83 |
| | | | | | | 40.48±4.36 | 14.39**±6.02 | 126/139 |
| | | | | | | 40.68±3.99 | 44.54±13.28 | 118/119 |
| | | | | | | 50.00±7.91 | 36.90±14.49 | 78/84 |
| | | | | | | 51.90±7.89 | 60.87±15.00 | 79/92 |
| | | | | | | 61.73±6.71 | 44.68*±6.96 | 81/94 |

*p < 0.05
**p < 0.005

**Table 11 Thermal resistance of RA 200 µM treated nematodes.** Effect of 200 µM RA on thermal resistance of N2 and four different mutant strains compared to respective untreated control (refers to Fig. 23 B).

| Genotype | Hours of heat stress | Survivors (%) all trials pooled ± SEM | | n Control/ Treated | Trials | Survivors (%) single trials ± SEM | | n Control/ Treated |
|---|---|---|---|---|---|---|---|---|
| | | Control (0µM) | Treated (RA 200 µM) | | | Control (0 µM) | Treated (RA 200 µM) | |
| N2 | 8 h | 59.64±3.45 | 68.54*±4.96 | 665/654 | 8 | 67.86±3.48 | 77.45±3.90 | 112/102 |
| | | | | | | 53.33±9.43 | 74.42±1.73 | 30/43 |
| | | | | | | 69.43±2.99 | 72.16±4.31 | 84/45 |
| | | | | | | 55.96±3.03 | 65.66±6.49 | 63/58 |
| | | | | | | 72.06±4.25 | 77.86±2.14 | 111/118 |
| | | | | | | 45.28±4.35 | 35.21±4.89 | 115/124 |
| | | | | | | 50.66±9.16 | 74.75**±5.96 | 86/102 |
| | | | | | | 62.56±9.34 | 70.83±10.49 | 64/62 |
| *osr-1 (rm1)* | 7 h | 61.50±2.85 | 65.64±2.75 | 205/216 | 2 | 58.63±4.50 | 66.33±3.32 | 108/113 |
| | | | | | | 64.37±2.32 | 64.94±4.89 | 97/103 |
| *sek-1 (ag1)* | 6 h | 44.06±9.53 | 43.13±11.83 | 49/53 | 3 | 25.00±0.00 | 22.22±0.00 | 8/9 |
| | | | | | | 53.85±0.00 | 44.00±0.00 | 26/25 |
| | | | | | | 53.33±0.00 | 63.16±0.00 | 15/19 |
| *sir-2.1 (ok434)* | 7 h | 63.45±3.92 | 71.43±4.15 | 279/184 | 3 | 55.95±1.67 | 73.15*±4.70 | 84/108 |
| | | | | | | 61.86±3.34 | 64.71±8.38 | 97/85 |
| | | | | | | 71.84±10.63 | 78.82±6.81 | 98/91 |
| *unc-43 (n498n1186)* | 8 h | 54.67±5.56 | 56.84±8.35 | 342/320 | 3 | 46.94±7.71 | 56.25±1.78 | 98/32 |
| | | | | | | 40.48±4.36 | 30.72±4.06 | 126/153 |
| | | | | | | 76.60±3.99 | 83.93±4.79 | 118/135 |

*p < 0.05
**p < 0.005

## 3.7 Global transcriptional profiling: DNA-microarray results from Q treated nematodes

To gain a deeper insight of how Q deploys its action by genetic regulation *in vivo*, global transcriptional profiling has been performed using DNA-microarrays. Q has been applied in three different concentrations: Q 50, 100 and 200 µM. By investigation of this concentration range (spanning from pre-lifespan extending 50 µM, towards the life extending concentrations Q 100 µM and 200 µM), it is possible to determine dose-dependent differences in the transcriptional output. By pooling the commonly regulated genes of the life extending concentrations on one hand ($Q_{longevity}$) and pooling of commonly regulated genes of all three concentrations on the other hand ($Q_{all}$), it was furthermore possible to curtail life prolonging transcriptional outputs. Unfortunately it was not possible to include an already toxic concentration, since at doses above 200 µM Q the compound did not solve completely and aggregated in the agar.

The whole dataset, with fold change values for all 22 548 transcripts and indicated significance can be found online in an excel spreadsheet (Online Supplementary 2) under following web address:
https://docs.google.com/leaf?id=0B-ebpwy-wEV6MNGQ1MzU1ZGYtYjZhYS00ZGY2LWIwNTYtMjM5MGE1M2ZhMzJi&hl=en_US&authkey=CNuBhcgP

In addition to Q treatment, this spreadsheet contains DNA-microarray datasets from the literature which characterized various lifespan-prolonging conditions and will be discussed in the following sections. Genes belonging to gene expression mountains (according to Kim et al., 2001), as well as gene-classes and –groups are highlighted in the list ;thus, it is possible to filter for various conditions and gene categories with the autofilter function in microsoft excel, implying that the array analyses described in the following sections can be reproduced. For a short manual how to use the filter function in the Online Supplementary refer to the Supplementary information 1 in the Appendix.

### 3.7.1 Transcriptional profiles

Initial analysis of gene expression profiles using Venn diagrams (Fig. 24) revealed that gene expression patterns at different Q doses mirrored longevity effects: the differential regulated genes (DEGs), up- or downregulated more than 1.25-fold in Q 50 µM treated nematodes, differ strongly from the DEGs in Q 100 and 200 µM animals. In contrast, the expression profiles in response to the two latter concentrations resemble each other in the magnitude as well as in the majority of regulated transcripts. The total number of DEGs increase in Q 100 µM compared to Q 50 µM exposed *C. elegans* up to 3.3 fold, however, the increment in Q 200 µM surpass Q 100 µM treated nematodes just about marginally 1.1 fold. A further discrepancy between the non-longevity provoking concentration Q 50 µM and both longevity-mediating ones, is the proportion of up- and down- regulated transcripts. Whereas in Q 50 µM three times more genes are up- compared to down- regulated, the ratio reverses in the higher concentrations, where approximately two times (2 fold in Q 100 µM and 1.7 fold in Q 200 µM) more genes were repressed contrasting to induced ones. Furthermore, the high percentage of exclusively Q 50 µM regulated transcripts (37%) points to a distinct mode of action, compared to the higher doses, which presumably lead to the observed variance in life extension.

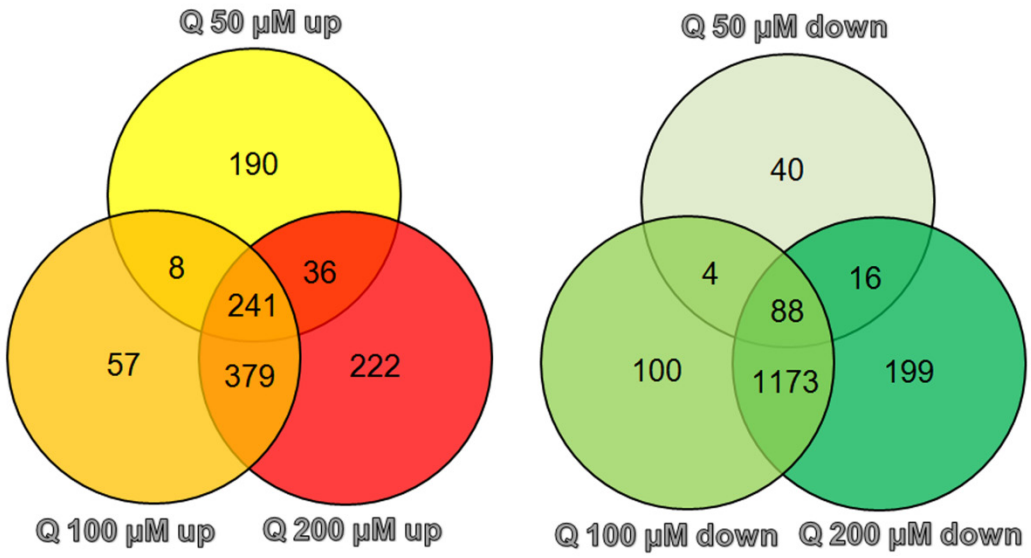



**Fig. 24 Number of significantly up- and down-regulated genes due to Q 50, 100 and 200 µM.** Overview of significant DEGs (fold change > 1.25 or < 0.8) in response to Q 50, 100, 200 µM. Venn diagram showing the overlap of significantly either up- or down-regulated genes per concentration.

### 3.7.2 Gene Ontology (GO) Analysis

DEGs were classified using gene ontology terms to obtain functional insights into gene expression responses. Figures 25, 26 and 27 depict Gene Ontology trees (GO-trees) computed from DEGs; both up- and down-regulated genes were analyzed together, in Q 50, 100 and 200 µM treated nematodes, respectively. To keep better clarity these GO-trees are restricted to superordinated topics. While Q 50 µM affects solely anatomical structure morphogenesis (developmental process) and structural molecule activity, Q 100 and 200 µM provoke a broad response in many fields, e.g. regulation of growth, reproduction, locomotion, cellular processes, developmental processes (like embryonic development, larval development and also body morphogenesis), nucleotide binding processes, as well as the formation of cell junctions. Importantly, only marginal differences are observed between both Q concentrations, reflecting the similar positive impact on lifespan.

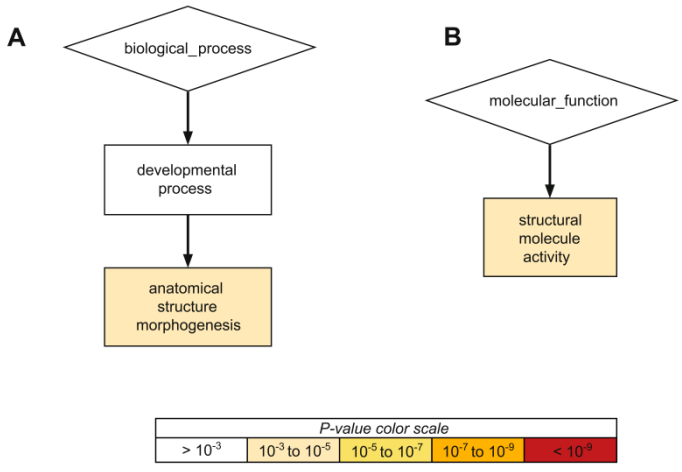



**Fig. 25 Gene Ontology trees for overrepresented GO-terms in Q 50 µM treated nematodes** out of the categories **(A)** Biological Process and **(B)** Molecular Function.

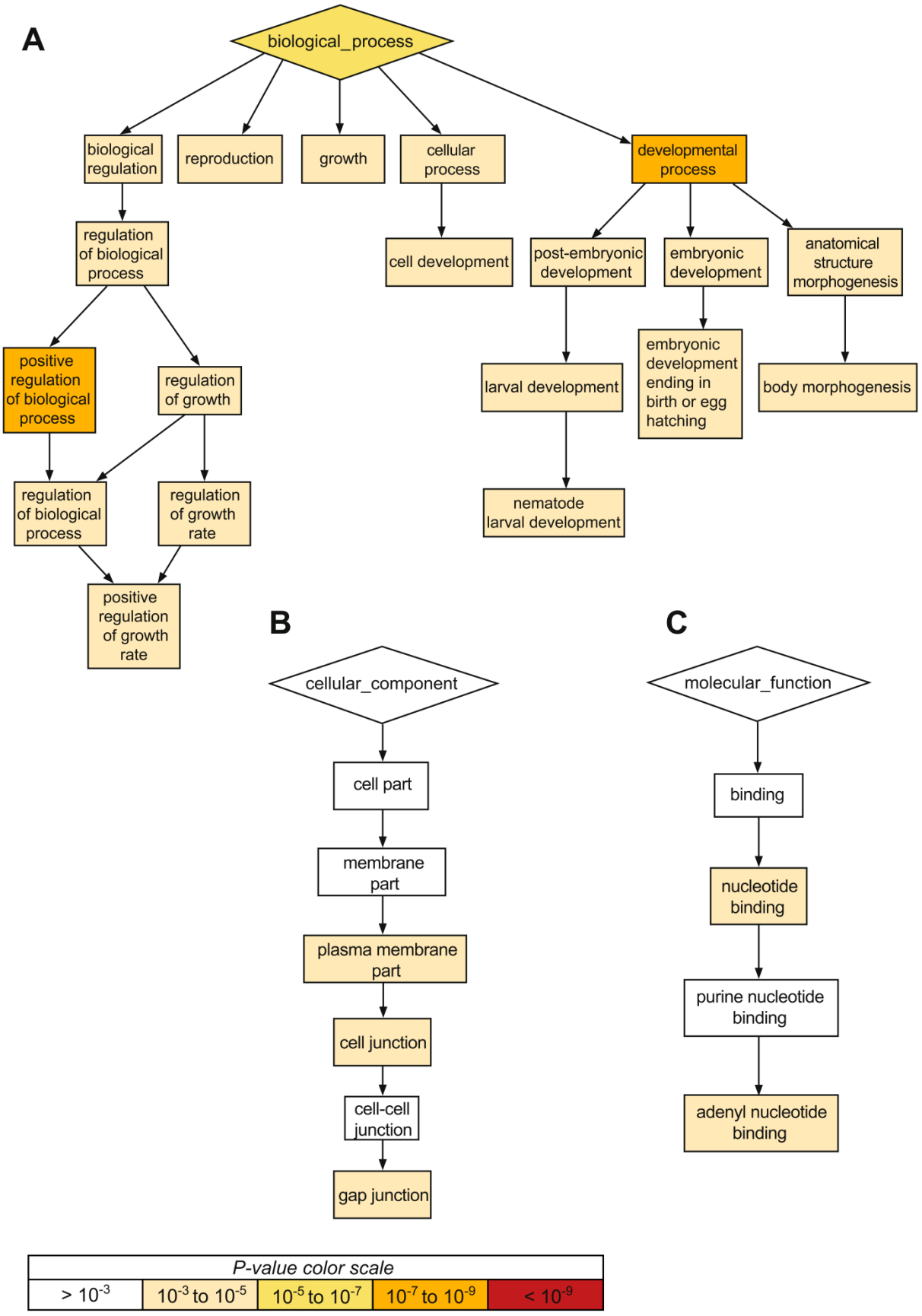



**Fig. 26 Gene Ontology trees for overrepresented GO-terms in Q 100 µM treated nematodes** out of the categories **(A)** Biological Process, **(B)** Cellular Component and **(C)** Molecular Function.

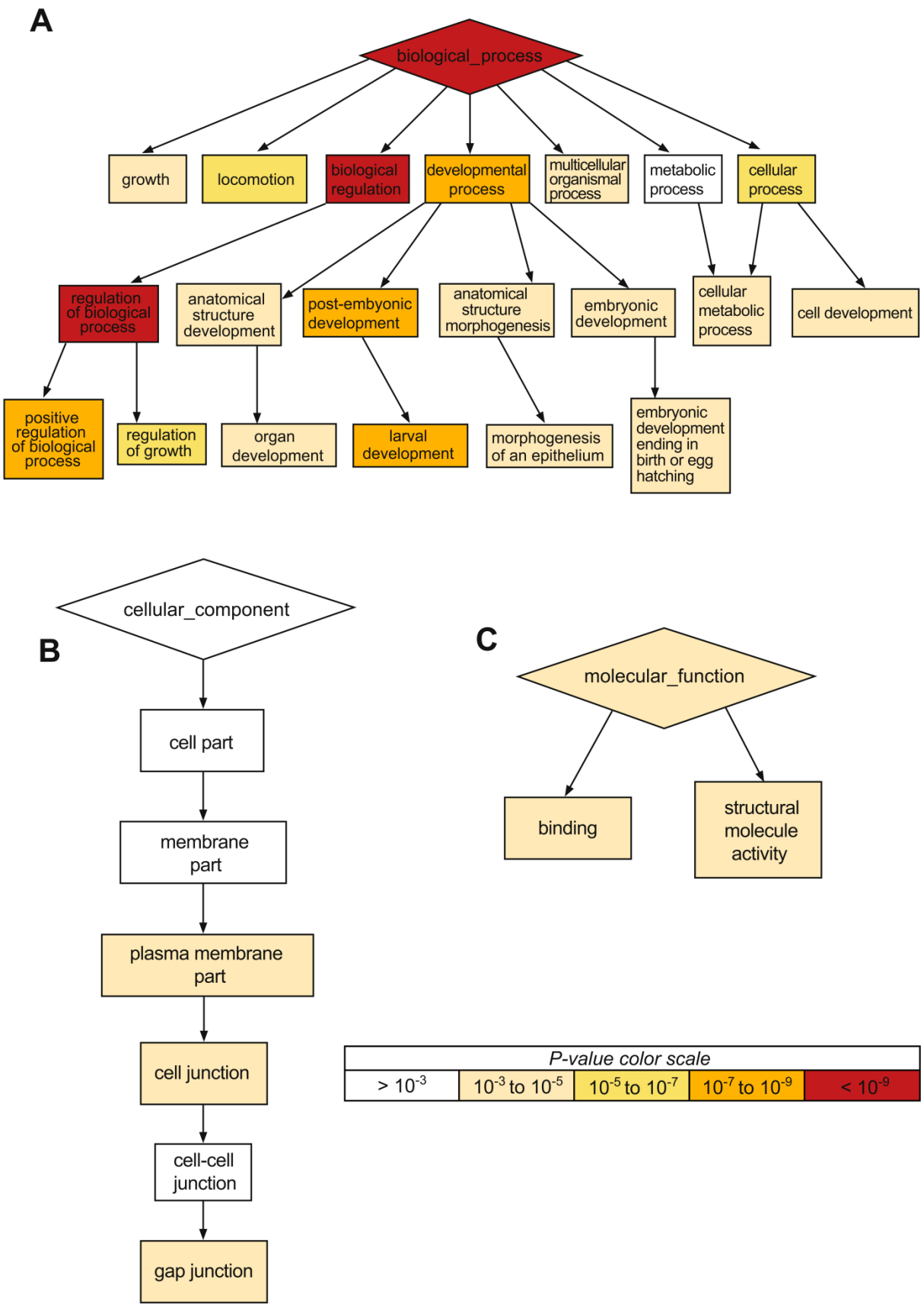



**Fig. 27 Gene Ontology trees for overrepresented GO-terms in Q 200 µM treated nematodes** out of the categories **(A)** Biological Process, **(B)** Cellular Component and **(C)** Molecular Function.

To further analyze the functional overlap between the single concentrations, commonly regulated GO-terms have been determined. The Venn diagram in Fig. 28 shows the number of exclusive and overlapping GO-categories in all three treatments (again concerning up- and down-regulated genes together). A few GO categories are overrepresented in all three concentrations (e.g. body morphogenesis, oxidation reduction, oxidoreductase activity, DNA packaging, protein-DNA-assembly, nucleosome organization and assembly, chromatin assembly, monocarboxylic acid metabolic process, cuticle development, and cytoskeleton etc; cf. Supplementary Table 2). Comparing the mutual overlap between the Q concentrations, a strikingly similar regulation of GO categories in lifespan prolonging conditions Q 100 and 200 µM can be observed. In contrast, the longevity-ineffective Q 50 µM concentration regulates only a few GO terms and shows little overlap with either Q 100 µM or Q 200 µM. Hence, longevity promoting concentrations of Q induce longevity-specific, broadly overlapping gene expression responses as assessed by GO terms.

Nevertheless, some GO-terms differ also between both lifespan prolonging concentrations: One term (among others) which can just exclusively be found in Q 100 µM is "determination of adult lifespan" (genes in this term are: *tir-1, ifta-2, ceh-20, gei-7, sod-3, dod-20, fkb-3, dod-22, unc-112, dod-23, acdh-1, spp-1, cyp-35b1, cln-3.3, vha-12, che-11, alp-1, ftn-1, jnk-1, sodh-1, dod-19, aqp-1, ife-2, elt-1, nuc-1, cyp-34a9, dod-3, unc-52, ifc-2, thn-1, elo-2, mdt-15*). The involvement of many *dod*-genes (means downstream of DAF-16) and *sod-3*, as well as *sodh-1* which are also regulated by DAF-16, would point towards an involvement of this central FOXO-transcription factor in Q induced logevity. However, it is not clear whether the observed difference between Q 100 µM and Q 200 µM is physiologically relevant or an experimental artifact that arises from technical variability in the array measurements. Of note, the putatively central "determination of adult life span" genes do not show up in the set of predicted aging-related genes, $Q_{longevity}$, which is defined based on overlaps of Q 100 µM and Q 200 µM. Additional information about regulated genes in all GO-terms of single concentrations can be found in Online Supplementary 1 and downloaded online under following link:
https://docs.google.com/leaf?id=0B-ebpwywEV6MNGIzODk4YWItMzYwNS00MTdjLWFmODEtNDI3NmYzOGUyMzcz&hl=en_US&authkey=CMDNr-8I

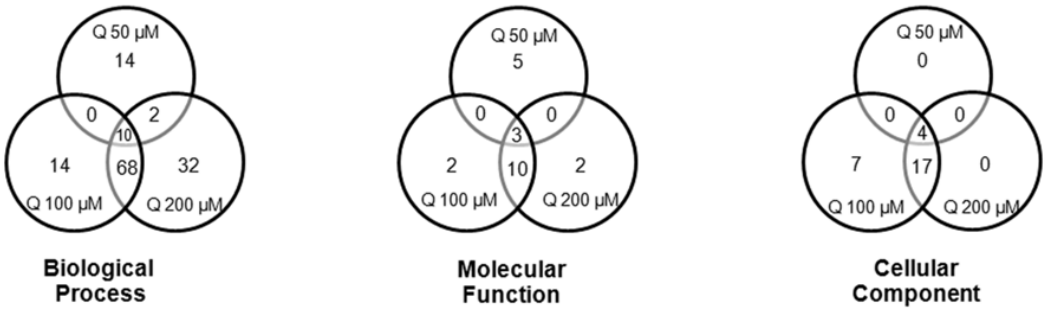



**Fig. 28 Overlap of overrepresented GO-categories in single Q concentrations.** GO terms were determined with DAVID software ($p < 0.05$, fold-change of statistically significant genes to reference control > 1.25 or < 0.8). Additional information about terms numbers and names can be found in Supplementary Table 2 and the Online Supplementary 1.

To gain further insights into the mechanistic basis of the Q longevity effect, a cluster analysis of GO-terms was performed for different concentrations of Q. A cluster analysis groups GO-terms with similar inclosed genes, thereby revealing redundancies and allowing for complexity reduc-

tion of the GO analysis output. Accordingly, only the most significant term of each GO cluster is given in the summary of the GO cluster analysis results (Tables 12 and 13). Since Q 100 and 200 µM induce largely similar transcriptional responses, overlapping genes were pooled to a new group called $Q_{longevity}$, and the GO clustering analysis focused on a comparison of $Q_{longevity}$ with the pre-life extension dose Q 50 µM (see Supplementary Tables 3 and 4 for Q 100 and 200 µM results). Note that up- and down-regulated genes were treated separately and that slightly different results would be obtained when grouping together positively and negatively responding genes. The putatively lifespan-extending up-regulated transcripts in $Q_{longevity}$ contained, among others, the following overrepresented GO-terms: (i) chromatin assembly, (ii) lipid metabolic process, (iii) monooxygenase activity and (iv) nucleosome. GO-terms revealed from down-regulated genes included (i) nervous system development, (ii) regulation of muticellular organism growth, (iii) dauer entry, regulation (iv) -of transcription, (v) - of response to stimulus, (vi) -of cell communication, (vii) -of biological quality, (viii) -locomotion and (ix) -programmed cell death. All of these terms point towards a profund impact on signaling and development common to both longevity promoting concentrations. Moreover, the longevity-related transcriptional processes resemble parts of the dauer program. GO-terms in Q 50 µM revealed among others lysozyme activity and oxidoreductase activity acting on the CH-CH group of donors, what points toward an upgraded immunity and stress resistance, also in this pre-life prolonging concentration.

**Table 12 GO cluster analysis with significantly up- or down- regulated transcripts in Q 50 µM.** Displayed is one significant GO-term per cluster. (DAVID, adjusted on "medium classification stringency", $p < 0.05$).

| GO-term | No. DEGs | No. genes in GO-term | p-value | Fold Enrichment |
|---|---|---|---|---|
| **Up-regulated overrepresented GO-terms in Q 50 µM** | | | | |
| **Biological Process** | | | | |
| GO:0006082~organic acid metabolic process | 17 | 217 | 2,13E-05 | 3,55E+00 |
| GO:0008152~metabolic process | 120 | 3776 | 7,77E-08 | 1,44E+00 |
| GO:0040002~collagen and cuticulin-based cuticle development | 6 | 89 | 4,57E-02 | 3,05E+00 |
| GO:0006725~cellular aromatic compound metabolic process | 7 | 77 | 6,75E-03 | 4,12E+00 |
| GO:0008610~lipid biosynthetic process | 8 | 104 | 7,79E-03 | 3,49E+00 |
| GO:0031497~chromatin assembly | 4 | 32 | 3,24E-02 | 5,66E+00 |
| GO:0010171~body morphogenesis | 20 | 582 | 5,28E-02 | 1,56E+00 |
| **Cellular Coponent** | | | | |
| GO:0016021~integral to membrane | 145 | 6596 | 1,35E-02 | 1,11E+00 |
| GO:0000786~nucleosome | 4 | 30 | 2,06E-02 | 6,74E+00 |
| **Molecular Function** | | | | |
| GO:0003796~lysozyme activity | 4 | 8 | 8,18E-04 | 1,97E+01 |
| GO:0016627~oxidoreductase activity, acting on the CH-CH group of donors | 9 | 67 | 2,71E-04 | 5,28E+00 |
| GO:0051213~dioxygenase activity | 4 | 20 | 1,33E-02 | 7,87E+00 |
| GO:0016878~acid-thiol ligase activity | 3 | 8 | 1,62E-02 | 1,47E+01 |
| GO:0016746~transferase activity, transferring acyl groups | 9 | 162 | 5,35E-02 | 2,18E+00 |
| **Down-regulated overrepresented GO-terms in Q 50 µM** | | | | |
| **Biological Process** | | | | |
| GO:0045449~regulation of transcription | 16 | 817 | 5,65E-03 | 2,15E+00 |
| GO:0007399~nervous system development | 6 | 107 | 2,71E-03 | 6,14E+00 |
| GO:0050789~regulation of biological process | 43 | 3457 | 8,69E-03 | 1,36E+00 |
| **Cellular Component** | | | | |
| GO:0005622~intracellular | 31 | 3047 | 4,41E-02 | 1,35E+00 |
| **Molecular Function** | | | | |
| GO:0043167~ion binding | 26 | 1966 | 2,51E-02 | 1,50E+00 |
| GO:0043565~sequence-specific DNA binding | 11 | 473 | 7,61E-03 | 2,65E+00 |

**Table 13 GO cluster analysis with significantly up- or down- regulated transcripts in $Q_{longevity}$.** Displayed is one significant GO-term per cluster. The cluster analysis of GO-terms in single concentrations can be found in Supplementary Tables 3 and 4. (DAVID, adjusted on "medium classification stringency", $p < 0.05$).

| GO-term | No. DEGs | No. genes in GO-term | p-value | Fold Enrich-ment |
|---|---|---|---|---|
| **Up-regulated overrepresented GO-terms in $Q_{longevity}$** | | | | |
| **Biological Process** | | | | |
| GO:0031497~chromatin assembly | 7 | 32 | 8,42E-05 | 9,28E+00 |
| GO:0006629~lipid metabolic process | 17 | 309 | 2,42E-03 | 2,33E+00 |
| GO:0006636~unsaturated fatty acid biosynthetic process | 3 | 7 | 1,06E-02 | 1,82E+01 |
| GO:0019400~alditol metabolic process | 3 | 15 | 4,70E-02 | 8,49E+00 |
| GO:0009451~RNA modification | 4 | 25 | 2,00E-02 | 6,79E+00 |
| GO:0008152~metabolic process | 105 | 3776 | 1,65E-02 | 1,18E+00 |
| **Cellular Component** | | | | |
| GO:0000786~nucleosome | 7 | 30 | 7,41E-05 | 9,47E+00 |
| GO:0016021~integral to membrane | 175 | 6596 | 4,74E-02 | 1,08E+00 |
| GO:0005856~cytoskeleton | 15 | 235 | 1,93E-03 | 2,59E+00 |
| **Molecular Function** | | | | |
| GO:0016788~hydrolase activity, acting on ester bonds | 26 | 467 | 1,32E-03 | 1,99E+00 |
| GO:0004497~monooxygenase activity | 11 | 107 | 7,93E-04 | 3,67E+00 |
| **Down-regulated overrepresented GO-terms $Q_{longevity}$** | | | | |
| **Biological Process** | | | | |
| GO:0007399~nervous system development | 35 | 107 | 8,00E-15 | 4,77E+00 |
| GO:0007155~cell adhesion | 21 | 64 | 4,16E-09 | 4,79E+00 |
| GO:0006928~cell motion | 27 | 98 | 1,06E-09 | 4,02E+00 |
| GO:0009653~anatomical structure morphogenesis | 124 | 1012 | 3,41E-11 | 1,79E+00 |
| GO:0040012~regulation of locomotion | 32 | 224 | 1,24E-04 | 2,09E+00 |
| GO:0051239~regulation of multicellular organismal process | 71 | 635 | 3,41E-05 | 1,63E+00 |
| GO:0065007~biological regulation | 290 | 3514 | 1,70E-05 | 1,20E+00 |
| GO:0007167~enzyme linked receptor protein signaling pathway | 9 | 32 | 1,11E-03 | 4,10E+00 |
| GO:0018991~oviposition | 41 | 287 | 1,10E-05 | 2,09E+00 |
| GO:0045137~development of primary sexual characteristics | 16 | 102 | 3,78E-03 | 2,29E+00 |
| GO:0001667~ameboidal cell migration | 6 | 12 | 7,78E-04 | 7,30E+00 |
| GO:0050794~regulation of cellular process | 156 | 1626 | 2,79E-06 | 1,40E+00 |
| GO:0009887~organ morphogenesis | 42 | 377 | 1,94E-03 | 1,63E+00 |
| GO:0043053~dauer entry | 7 | 28 | 1,03E-02 | 3,65E+00 |
| GO:0014018~neuroblast fate specification | 3 | 3 | 1,34E-02 | 1,46E+01 |
| GO:0031344~regulation of cell projection organization | 7 | 24 | 4,64E-03 | 4,26E+00 |
| GO:0008361~regulation of cell size | 8 | 28 | 2,26E-03 | 4,17E+00 |
| GO:0006357~regulation of transcription from RNA polymerase II promo-ter | 13 | 85 | 1,25E-02 | 2,23E+00 |
| GO:0048583~regulation of response to stimulus | 12 | 67 | 5,36E-03 | 2,61E+00 |
| GO:0010646~regulation of cell communication | 19 | 152 | 1,56E-02 | 1,82E+00 |
| GO:0065008~regulation of biological quality | 27 | 214 | 2,93E-03 | 1,84E+00 |
| GO:0045185~maintenance of protein location | 5 | 16 | 2,04E-02 | 4,56E+00 |
| GO:0042464~dosage compensation, by hypoactivation of X chromo-some | 4 | 9 | 1,96E-02 | 6,49E+00 |
| GO:0048523~negative regulation of cellular process | 18 | 132 | 8,37E-03 | 1,99E+00 |
| GO:0043067~regulation of programmed cell death | 8 | 46 | 3,53E-02 | 2,54E+00 |
| GO:0043067~regulation of programmed cell death | 8 | 46 | 3,53E-02 | 2,54E+00 |
| GO:0050793~regulation of developmental process | 26 | 228 | 1,27E-02 | 1,66E+00 |
| GO:0007154~cell communication | 14 | 107 | 3,03E-02 | 1,91E+00 |
| **Cellular Component** | | | | |
| GO:0044459~plasma membrane part | 36 | 256 | 1,06E-06 | 2,47E+00 |
| GO:0005622~intracellular | 209 | 3047 | 6,71E-04 | 1,20E+00 |
| GO:0030054~cell junction | 18 | 124 | 6,19E-04 | 2,55E+00 |
| GO:0005882~intermediate filament | 5 | 12 | 3,54E-03 | 7,32E+00 |
| GO:0044421~extracellular region part | 8 | 44 | 1,13E-02 | 3,19E+00 |
| GO:0005788~endoplasmic reticulum lumen | 6 | 11 | 2,02E-04 | 9,58E+00 |
| GO:0005856~cytoskeleton | 21 | 235 | 4,58E-02 | 1,57E+00 |
| **Molecular Function** | | | | |
| GO:0030528~transcription regulator activity | 76 | 685 | 1,01E-05 | 1,66E+00 |
| GO:0016864~intramolecular oxidoreductase activity, transposing S-S bonds | 3 | 4 | 2,44E-02 | 1,12E+01 |
| GO:0016706~oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors | 4 | 9 | 1,84E-02 | 6,65E+00 |

### 3.7.3 Overrepresented KEGG pathways

GO-terms provide useful information about biological functions, but often do not reveal comprehensive molecular mechanisms of action. DEGs (both up- and down-regulated together) were mapped on KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways to assign them to intracellular regulatory pathways. Table 14 gives an overview of overrepresented KEGG pathways in all three single concentrations and additionally for pooled datasets of commonly regulated genes in $Q_{longevity}$ and commonly regulated DEGs from all three concentrations ($Q_{all}$). The 5 overrepresented KEGG pathways in Q 50 µM clasps exclusively metabolic pathways, and mainly comprise induced DEGs (amino acid-, lipid- and xenobiotic – metabolism) (Table 15). In Q 100 and 200 µM, nine and eight KEGG pathways are overrepresented respectively, a big proportion (seven pathways) of which are overlapping as expected ($Q_{longevity}$). Besides metabolic pathways (amino acid-, lipid- and xenobiotic/drug – metabolism), also cellular processes (transport & catabolism, lysosome), as well as environmental information processing (Signal transduction, Wnt- and TGF-beta- signaling) are affected. The fold change values for individual regulated DEGs in each KEGG-pathway from $Q_{longevity}$ are shown in Table 16. (Q 100 µM, Q 200 µM and $Q_{all}$ can be found in Supplementary Tables 5, 6 and 7). $Q_{longevity}$ DEGs from arginin- and prolin-metabolism, as well as from the lysosome are up- as well as down-regulated. Genes of the TGF-beta- and Wnt-signaling pathway (both pathways include partly the same genes) are prevailingly repressed, whereas genes represented in drug metabolism and metabolism of xenobiotics by cytochrome P450 are predominantly induced (Table 16). In the following a few aspects from this KEGG pathway analysis will be deciphered in closer detail.

All proteins, and thus all enzymes and structural elements in living things are composed of amino acids. Therefore the modulation of their metabolism is one the first steps to provoke changes in all kind of cellular processes. $Q_{longevity}$ obviously interferes with arginine and proline, as well as with cysteine and methionine metabolism. Enhanced levels of arginine, for example, have been linked to an improved cellular immune response (Fürst et al., 2004; Landmesser, 2004). Furthermore, the thiol side chain of cysteine is susceptible to oxidation (thereby possess antioxidative properties) and often participates in enzymic reactions as a nucleophil (http://en.wikipedia.org/wiki/Cysteine). Cysteine partakes in numerous biological functions, e. g. as a precursor for the antioxidant gluthatione, in the formation of disulfide bonds (important for the cross-linking, folding and stability of extracellular proteins (Sevier et al, 2002)) and also in many post-translational modifications. Proline is important for the assembly of collagens. Since collagens are among the gene-groups which are highly overrepresented in Q treated nematodes (see chapter below), it is not suprising that prolin metabolism is affected. Interestingly, growing evidence from studies with methionine indicate that imbalance of methionine can lead to prolonged lifespan in some animals (Richard, 2009). Mice on a normal diet, but restricted for methionine, displayed enhanced lifespans (Miller et al., 2005). And application of methionine to Drosophila on a CR diet, restored egg-laying to normal, without reducing the lifespan (Grandison et al., 2009). Hence, altering the amino acid metabolism by Q provides broad possibilities to trigger the physiological status of the nematodes.

Lysosomes are membrane-delimited organelles in animal cells serving as the cell's main digestive compartment. All kind of macromolecules are delivered for degradation into this acidic (pH = 5) compartment, which contains more than 40 hydrolases (http://www.genome.jp/dbget-bin/www_bget?map04142). The fact that some of the genes belonging to lysosome-related processes are up- while others are down-regulated, suggests that certain degradation processes are selectively targeted following $Q_{longevity}$ treatment.

Wnt proteins are secreted morphogens that are involved in basic developmental processes, such as cell-fate specification, progenitor-cell proliferation and the control of asymmetric cell division. There are at least three different Wnt pathways: (i) the canonical pathway, (ii) the planar cell polarity (PCP) pathway and (iii) the Wnt/$Ca^{2+}$ pathway (http://www.genome.jp/dbget-bin/www_bget?pathway:cel04310). Since *jnk-1* (genetic player in the canonical pathway) is repressed due to $Q_{longevity}$ and *unc-43* (CaMKII) was found in mutant lifespan assays to be required for Q mediated life extension, suggests that the canonical pathway as well as the Wnt/$Ca^{2+}$ pathway are involved in $Q_{longevity}$s' action. Both provide valuable tools for modulating the lifespan of the nematodes.

Q also regulates signaling via the TGF-beta family of cytokines which control a wide spectrum of further cellular functions - such as proliferation, apoptosis, differentiation and migration TGF-beta family members bind to their cognate receptor in the plasma membrane, which then in turn triggers phosphorylation of Smad transcription factors. These translocate into the nucleus and induce specific genes through cooperative interactions with other DNA-binding- and coactivator- (or co-repressor-) proteins and thereby provoke various outputs.

All of these findings further underline the profound and complex impact of $Q_{longevity}$ *in vivo*. By modulation of basic signaling pathways, up-grading of the biotransformation system, influence on the metabolism of certain aminoacids, as well as the selective regulation of cellular degradation processes, Q provides the basis for the longevity phenotype in *C. elegans*.

**Table 14 KEGG pathways overview.** According DEGs with fold change values, gene annotation and enrichement values can be found in Table 15 (Q 50 µM), Table 16 ($Q_{longevity}$) and Supplementary Tables 5 (Q 100 µM), 6 (Q 200 µM) and 7 ($Q_{all}$).

| Conditon | KEGG pathway | No. DEGs/ listed genes | p-value |
|---|---|---|---|
| **Q 50 µM** | cel00330: Arginine and proline metabolism | 6/30 | 0.002 |
| | cel00062: Fatty acid elongation in mitochondria | 4/13 | 0.007 |
| | cel00480: Glutathione metabolism | 5/35 | 0.022 |
| | cel00270: Cysteine and methionine metabolism | 4/25 | 0.041 |
| | cel00980: Metabolism of xenobiotics by cytochrome P450 | 4/26 | 0.045 |
| **Q 100 µM** | cel04142: Lysosome | 15/68 | 0.000 |
| | cel04350: TGF-beta signaling pathway | 11/36 | 0.001 |
| | cel00600: Sphingolipid metabolism | 8/20 | 0.001 |
| | cel04310: Wnt signaling pathway | 14/60 | 0.001 |
| | cel00980: Metabolism of xenobiotics by cytochrome P450 | 8/26 | 0.005 |
| | cel00982: Drug metabolism | 8/29 | 0.009 |
| | cel00330: Arginine and proline metabolism | 8/30 | 0.011 |
| | cel00270: Cystein and methionine metabolism | 6/25 | 0.033 |
| | cel00350: Tyrosine metabolism | 5/19 | 0.047 |
| **Q 200 µM** | cel04350: TGF-beta signaling pathway | 13/36 | 0.000 |
| | cel04310: Wnt signaling pathway | 15/60 | 0.001 |
| | cel00982: Drug metabolism | 9/29 | 0.005 |
| | cel00480: Glutathione metabolism | 9/35 | 0.015 |
| | cel00330: Arginine and proline metabolism | 8/30 | 0.020 |
| | cel04142: Lysosome | 14/68 | 0.026 |
| | cel00980: Metabolism of xenobiotics by cytochrome P450 | 7/26 | 0.033 |
| | cel00340: Histidine metabolism | 3/9 | 0.048 |
| **$Q_{longevity}$** | cel00330: Arginine and proline metabolism | 9/30 | 0.000 |
| | cel04350: TGF-beta signaling pathway | 9/36 | 0.001 |
| | cel04310: Wnt signaling pathway | 11/60 | 0.004 |
| | cel00982: Drug metabolism | 7/29 | 0.008 |
| | cel04142: Lysosome | 11/68 | 0.009 |
| | cel00270: Cystein and methionine metabolism | 6/25 | 0.018 |
| | cel00980: Metabolism of xenobiotics by cytochrome P450 | 6/26 | 0.022 |
| **$Q_{all}$** | Cel00330: Arginine and proline metabolism | 4/19 | 0.005 |

| p < 1.0E-03 | p < 1.0E-02 | p < 5.0E-02 | p > 5.0E-02 |
|---|---|---|---|

**Table 15 Overrepresented KEGG pathways found in Q 50 µM treated nematodes.** Further summarized are the DEGs and statistical proven expression values (FC), p-values and the Fold Enrichment for Q 50 µM. The numbers in italics in Q 100 and 200 µM show FC values without guarantee of statistical robustness and are just shown to see variances in single concentrations.

| Overrepresented KEGG pathways in Q 50 µM | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel00330: Arginine and proline metabolism** | | | | | | |
| 0.002 | 6.261 | T01B11.2a | 1.48 | *1.06* | *1.20* | Predicted alanine-glyoxylate aminotransferase |
| | | *dpy-18* | 1.26 | *0.85* | *0.77* | prolyl 4-hydroxylase alpha subunit |
| | | F44G3.2 | 1.53 | *2.18* | *1.89* | Creatine kinases |
| | | *phy-2* | 1.33 | *0.71* | *0.71* | Prolyl 4-hydroxylase alpha subunit |
| | | *smd-1* | 2.50 | *0.76* | *0.58* | S-adenosylmethionine decarboxylase |
| | | T22H6.2 | 1.29 | *0.73* | *0.69* | 1-pyroline-5-carboxylate synthetase |
| **cel00062: Fatty acid elongation in mitochondria** | | | | | | |
| 0.007 | 9.633 | *ech-1* | 1.69 | *1.04* | *1.06* | C.elegans cDNA clone yk5g6 |
| | | *ech-8* | 1.82 | *1.16* | *1.00* | Hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase |
| | | *hacd-1* | 2.68 | *0.84* | *0.86* | 3-hydroxyacyl-CoA dehydrogenase |
| | | Y48A6B.9 | 1.96 | *1.01* | *1.20* | $Zn^{2+}$-binding dehydrogenase |
| **cel00480: Glutathione metabolism** | | | | | | |
| 0.022 | 4.472 | F26E4.12 | 1.37 | *0.97* | *1.02* | Glutathione peroxidase |
| | | *gst-10* | 1.29 | *0.99* | *1.28* | Glutathione S-transferase |
| | | *gst-38* | 0.58 | *0.64* | *1.23* | Glutathione S-transferase |
| | | *gst-7* | 1.25 | *0.98* | *0.94* | Glutathione S-transferase |
| | | T25B9.9 | 1.37 | *0.95* | *1.00* | 6-phosphogluconate dehydrogenase |
| **cel00270: Cysteine and methionine metabolism** | | | | | | |
| 0.041 | 5.009 | *sams-4* | 1.33 | *1.35* | *1.29* | S-Adenosyl Methionine Synthetase |
| | | F42D1.2 | 0.78 | *1.09* | *1.17* | Predicted alanine-glyoxylate aminotransferase |
| | | R03D7.1 | 1.60 | *1.24* | *1.22* | S-adenosylmethionine synthetase |
| | | *smd-1* | 2.50 | *0.76* | *0.58* | Tyrosine aminotransferase |
| **cel00980: Metabolism of xenobiotics by cytochrome P450** | | | | | | |
| 0.045 | 4.816 | *gst-10* | 1.29 | *0.99* | *1.28* | Glutathione S-transferase |
| | | *gst-38* | 0.58 | *0.64* | *1.23* | Glutathione S-transferase |
| | | *gst-7* | 1.25 | *0.98* | *0.94* | Glutathione S-transferase |
| | | *ugt-50* | 1.43 | *0.78* | *0.99* | UDP-glucuronosyltransferase |

| < 0.65 | < 0.8 | <…> | > 1.25 | > 2.5 |
|---|---|---|---|---|

**Table 16 Overrepresented KEGG pathways found in $Q_{longevity}$ treated nematodes.** Further summarized are the DEGs and statistical proven expression values (FC), p-values and the Fold Enrichment for $Q_{longevity}$. The numbers in italics in Q 50 µM show FC values without guarantee of statistical robustness and are just shown to see variances in single concentrations.

| Overrepresented KEGG pathways in $Q_{longevity}$ | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel00330: Arginine and Proline metabolism** | | | | | | |
| 0.0004 | 4.648 | *alh-1* | *1.14* | 1.45 | 1.42 | Aldehyde dehydrogenase 2 |
| | | C10C5.4 | *1.15* | 1.68 | 1.95 | Aminoacylase ACY1 and related metalloexopeptidases |
| | | T09B4.8 | *0.83* | 0.74 | 0.78 | aminotransferase |
| | | *dpy-18* | *1.09* | 0.69 | 0.62 | Prolyl 4-hydroxylase alpha subunit |
| | | F44G3.2 | *1.53* | 2.18 | 1.89 | Creatine kinases |
| | | F55G1.9 | *1.17* | 1.29 | 1.32 | Pyrroline-5-carboxylate reductase |
| | | *phy-2* | *1.33* | 0.71 | 0.71 | Prolyl 4-hydroxylase alpha subunit |
| | | *smd-1* | *2.40* | 0.71 | 0.59 | S-adenosylmethionine decarboxylase |
| | | T22H6.2a | *1.29* | 0.73 | 0.69 | 1-pyroline-5-carboxylate synthetase |
| **cel04350: TGF-beta signaling pathway** | | | | | | |
| 0.0010 | 3.874 | *daf-1* | *0.89* | 0.79 | 0.73 | abnormal DAuer Formation |
| | | *skr-10* | *0.99* | 0.68 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12 / skr-13* | *1.01* | 0.63 | 0.58 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | *1.03* | 0.72 | 0.62 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | *1.04* | 0.80 | 0.67 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | *0.95* | 0.76 | 0.65 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | *0.78* | 0.73 | 0.54 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | *1.02* | 0.68 | 0.61 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *0.99* | 0.70 | 0.57 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-9* | *0.95* | 0.67 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| **cel04310: Wnt signaling pathway** | | | | | | |
| 0.0037 | 2.841 | *cwn-2* | *0.85* | 0.75 | 0.65 | Wnt family of developmental regulators |
| | | *dsh-1* | *0.87* | 0.70 | 0.63 | dishevelled-like protein |
| | | *jnk-1* | *0.71* | 0.74 | 0.62 | MAP kinase |
| | | *skr-10* | *0.99* | 0.68 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12 / skr-13* | *1.01* | 0.63 | 0.58 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | *1.03* | 0.72 | 0.62 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | *1.04* | 0.80 | 0.67 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | *0.95* | 0.76 | 0.65 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | *0.78* | 0.73 | 0.54 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | *1.02* | 0.68 | 0.61 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *0.99* | 0.70 | 0.57 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-9* | *0.95* | 0.67 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| **cel00982: Drug metabolism** | | | | | | |
| 0.0083 | 3.740 | *alh-5* | *0.73* | 1.95 | 2.28 | Aldehyde dehydrogenase |
| | | *fmo-2* | *0.69* | 3.22 | 4.47 | Flavin-containing monooxygenase |
| | | *gst-4* | *1.97* | 1.72 | 1.97 | Glutathione S-transferase |
| | | *gst-5* | *1.13* | 1.27 | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | *1.78* | 2.32 | 1.83 | Alcohol dehydrogenase, class V |
| | | *ugt-58* | *0.82* | 1.46 | 1.44 | UDP-glucuronosyl and -glucosyl transferase |
| | | *ugt-50* | *0.89* | 0.71 | 0.60 | UDP-GlucuronosylTransferase |

**Table continues on page 77**

**Continuation of Table 16**

| | | | | | | |
|---|---|---|---|---|---|---|
| **cel04142: Lysosome** | | | | | | |
| 0.0092 | 2.507 | *apm-3* | 0.86 | 0.75 | 0.65 | Clathrin-associated protein medium chain |
| | | *asm-2* | 1.20 | 1.38 | 1.79 | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | C33G3.4 | 0.89 | 0.73 | 0.68 | Predicted beta-mannosidase |
| | | *cpr-2* | 1.56 | 5.83 | 8.28 | Cysteine proteinase Cathepsin L |
| | | *cpr-3* | 0.70 | 0.64 | 0.73 | Cysteine proteinase Cathepsin L |
| | | F32H5.1 | 1.10 | 1.57 | 1.92 | Cysteine proteinase Cathepsin L |
| | | *mig-23* | 0.88 | 0.73 | 0.78 | Nucleoside phosphatase |
| | | *vha-15* | 0.87 | 0.77 | 0.75 | Vacuolar H+-ATPase V1 sector, subunit H |
| | | W07B8.4 | 2.61 | 3.57 | 4.11 | thiol protease; C,elegans cDNA clone yk150b4 |
| | | Y4C6B.6 | 1.42 | 1.30 | 1.57 | Beta-glucocerebrosidase |
| | | Y40D12A.2 | 1.33 | 0.63 | 0.71 | Serine carboxypeptidases (lysosomal cathepsin A) |
| **cel00270: Cysteine and methionine metabolism** | | | | | | |
| 0.0183 | 3.719 | T09B4.8 | 0.83 | 0.74 | 0.78 | aminotransferase |
| | | C06E7.3 | 1.33 | 1.35 | 1.29 | S-adenosylmethionine synthetase |
| | | *ldh-1* | 1.14 | 0.78 | 0.70 | Lactate dehydrogenase |
| | | *smd-1* | 2.40 | 0.72 | 0.59 | S-adenosylmethionine decarboxylase |
| | | *tag-32* | 3.32 | 2.57 | 4.18 | S-adenosylmethionine synthetase |
| | | ZC373.1 | 0.67 | 0.63 | 0.64 | Cystathionine beta-synthase and related enzymes |
| **cel00980: Metabolism of xenobiotics by cytochrome P450** | | | | | | |
| 0.0216 | 3.576 | *alh-5* | 0.73 | 1.95 | 2.28 | Aldehyde dehydrogenase |
| | | *gst-4* | 1.97 | 1.72 | 1.97 | Glutathione S-transferase |
| | | *gst-5* | 1.13 | 1.27 | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | 1.78 | 2.32 | 1.83 | Alcohol dehydrogenase, class V |
| | | *ugt-58* | 0.82 | 1.46 | 1.44 | UDP-glucuronosyl and -glucosyl transferase |
| | | *ugt-50* | 0.89 | 0.71 | 0.60 | UDP-GlucuronosylTransferase |

| < 0.65 | < 0.8 | <...> | > 1.25 | > 2.5 |
|---|---|---|---|---|

### 3.7.4 Selected gene-groups and -classes

Besides functional annotation by KEGG or GO analyses, it is important to identify groups of genes in order to gain insights into mechanistic principles governing global gene expression responses. To find specific gene classes regulated by each concentration of Q, the DEGs in Q 50, 100, 200 µM, $Q_{longevity}$ and $Q_{all}$ were matched to known gene-classes and –groups which were manually arranged by gene names or taken from Kim et al. (2001). Table 17 shows significant RFs of gene groups in which at least one dataset displays overrepresentation. Several gene classes are overrepresented in all single conditions and thus also in both pooled datasets ($Q_{all}$, $Q_{longevity}$): cell-adhesion and –structure, male- and sperm-enriched, *msps*, proteases, amino acid and lipid metabolism, collagens, and histone genes. The following gene classes appear to show distinct expression patterns for pre-life extension and life extension concentrations, and thus point to longevity-specific regulation: *cyp*-genes are overrepresented in all datasets, but not significant in Q 50 µM. *gst*-genes are overrepresented in all datasets except $Q_{all}$, which points towards distinct *gst*-genes in Q 50 µM and the both lifespan prolonging concentrations. Lysozymes are overrepresented in Q 50 and 100 µM as well in $Q_{all}$ (*lys-4*, *-2*, *-10*). Striking is the contrary lysozyme regulation in Q 50 µM (up) compared to the longevity mediating concentrations Q 100 and 200 µM (down). As also shown by the KEGG pathway analysis, genes for fatty acid oxidation, which are contained to a big extent in the groups of lipid metabolism, biosynthesis and dehydrogenases, are exclusively overrepresented in Q 50 µM. The striking difference between the longevity mediating concentrations Q 100 and 200 µM (and thus in $Q_{longevity}$) on one hand and Q 50 µM on the other hand, obviously lays in the exclusive

**Table 17 Representation Factors of overrepresented gene-groups and -classes** in Q 50, 100 and 200 µM treated nematodes and commonly regulated DEGs in pooled data sets of $Q_{longevity}$ and $Q_{all}$. Listed are only groups, in which at least one condition display significant overrepresentation (RF > 1). Dark grey fields mark groups, where all conditions are enriched; bright grey fields underline exclusive overrepresented groups in Q 100 and 200 µM, $Q_{longevity}$. Non-marked fields with RFs display diffuse enrichment. Additional information in Supplementary Table 8. *p < 0.05, **p < 0.005, ***p < 0.001.

| Gene-class or -group | Q 50 µM | Q 100 µM | Q 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|
| **amino acid metabolism** | 3.00 ** | 1.50 * | 1.60 * | 1.57 * | 2.44 * |
| **aminoxidase** | | | 3.10 * | | |
| **biosynthese** | 2.00 *** | 1.30 * | 1.20 * | 1.09 * | |
| **cell adhesion** | 4.10 ** | 2.90 *** | 2.50 *** | 3.18 *** | 4.51 * |
| **cell structure** | 5.70 *** | 3.10 *** | 2.90 *** | 3.26 *** | 7.83 *** |
| **collagen** | 6.20 *** | 2.40 *** | 2.70 *** | 2.63 *** | 5.95 *** |
| ***cyps*** | | 1.90 * | 1.80 ** | 1.89 * | 3.13 * |
| **dehydrogenases** | 3.10 *** | 1.30 * | 1.40 * | | |
| **fatty acid oxidation** | 5.20 *** | | | | |
| ***gsts*** | 3.70 ** | 2.10 ** | 2.00 * | 1.76 * | |
| **histone genes** | 3.80 ** | 2.30 ** | 2.30 *** | 2.33 ** | 5.34 *** |
| ***hsps*/chaperones/alpha crystallins** | | | 1.80 * | | |
| **hydrolases** | | 1.60 * | | | |
| **lectins** | | 1.20 * | | | |
| **lipid metabolism** | 2.80 *** | 1.70 *** | 1.60 *** | 1.46 ** | 1.94 * |
| **lysozyme** | 11.30 *** | 4.30 ** | | | 13.84 ** |
| **male enriched** | 2.00 *** | 1.50 *** | 1.60 *** | 1.54 *** | 2.82 *** |
| **monooxygenases** | | | 2.70 * | | |
| ***msps*** | 12.90 *** | 6.00 *** | 5.40 *** | 6.56 *** | 21.21 *** |
| **genes with PDZ domains** | | 1.80 * | 1.60 * | 2.00 * | |
| **peroxidases** | 5.20 * | | 2.30 * | | |
| **phosphatases** | | | 1.30 * | | 1.78 * |
| **proteases** | 1.90 * | 1.60 * | 1.40 * | 1.45 * | 2.25 * |
| **sperm-enriched** | 2.80 *** | 1.50 *** | 1.70 *** | 1.58 *** | 4.49 *** |
| **transcription factors** | | 1.40 * | 1.50 *** | 1.51 ** | |
| ***ugts*** | | 2.00 * | | 1.90 * | |
| **vitellogenin** | | 5.60 *** | 6.00 *** | 5.58 *** | |

overrepresentation of *pdz* encoding genes (important for transport, localization and assembly of supramolecular signaling complexes; Harris and Lim, 2001), and the overrepresentation of transcription factors and vitellogenins in the longevity mediating concentrations. Taken together, the analysis reveals several longevity-specific gene expression classes which likely underlie the beneficial effects of Q (more information for additional gene-groups and -classes, RFs and statistics in single concentrations and pooled datasets in Supplementary Table 8). Fold Change values for single genes in each gene-class and –group can be viewed in respective filter condition in the Online Supplementary 2 under
https://docs.google.com/leaf?id=0B-ebpwywEV6MNGQ1MzU1ZGYtYjZhYS00ZGY2LWIwNTYtMjM5MGE1M2ZhMzJi&hl=en_US&authkey=CNuBhcgP
(The manual for the use of the filtertable in the Online Supplementary 2 can be found in Supplementary Information 1 on pp. 179 - 181).

### 3.7.5 Meta-analysis: comparison of global transcriptional patterns in $Q_{longevity}$ and selected datasets in the literature

To date, several DNA-microarray studies have been conducted to identify age-related transcriptional changes in the nematode *C. elegans* (summarized in Golden and Melov, 2007). A systematic comparison among these datasets that would potentially allow for the identification of common age-related pathways has not been performed so far. In this section, datasets from Q treated nematodes will be related to published expression profiles obtained in long-lived mutants, dauers and worms treated with another life-extending PP (resveratrol). Questions to be addressed include: (i) Which aging-related perturbations share similar molecular mechanisms and are thus functionally related? (ii) What are the genetic players and molecular pathways underlying the Q provoked longevity phenotype? As a control, the analysis will include studies addressing the gene expression changes during the *C. elegans* life-cycle. Moreover, gene expression in immune challenged nematodes will be related to Q studies to confirm the involvement of the innate immunity p38 MAPK pathway (e.g. by *sek-1*) in Q action, as suggested by results from mutant lifespan assays.

Table 18 gives an overview about DNA-microarray studies which will be analyzed in the following sections. The full comparison of all published array studies listed and all single concentrations of Q can be found in Supplementary Tables 9, 10 and 11. In the main text, the focus will lie on the comparison between studies marked in grey in Table 18 and the pooled datasets of lifespan prolonging concentrations, namely $Q_{longevity}$. Notably, the study by Evans et al. (2008) is a meta-analysis addressing the expression output of *daf-2* mutants (Murphy et al., 2003; McElwee et al., (2004); note: included are mutations in differing allels) and immune challenged nematodes (Shapira et al., 2006; Troemel et al., 2006). In this work, it was deemed adequate to show the comparison to the pooled meta-analysis data from this study, instead of showing comparison of single study results, since single results differ in marginal parts only (not shown).

For meta-analysis, array studies were analyzed for similarities at the level of (i) overrepresented gene expression mountains (according to Kim et al., 2001) which characterize the global patterns of a transcriptional response and (ii) DEGs, to identify conspicous genes. The significance of overlap between two gene lists has been assigned by calculating the representation factor (RF), and significances were determined using hypergeometric probability. The complete comparison can be reconstructed in the Online Supplementary 2.

**Table 18 DNA-microarray studies addressing age- and immune-related issues**, which have been compared to datasets obtained from Q treated nematodes. Grey fields mark studies on which will be responded in the comparison to the pooled dataset $Q_{longevity}$ in the following sections. Henceforth, to each study will be refered by the nomenclature in red. RF- and p- values for the comparison of all studies with Q 50, 100 and 200 µM, as well as $Q_{longevity}$ and $Q_{all}$, can be found in Supplementary Table 11.

| Reference / DNA-microarray study | Genotype / condition |
|---|---|
| **Studies addressing the age dependent expression changes in wild type *C. elegans*** | |
| **Hill et al. (2000)** | 8 ages, 2 of which were adult, pools used, single array per age;<br>**Output:** **life-cycle changing genes**; gene expression course during life cycle from oocytes until 2 weeks old adults; |
| **Lund et al. (2001)** | sterile mutant strains (*fer-15*, *spe-9*;*fer-15*, and *spe-9*;*emb-27*); 6 adult ages, pools used, three sterile mutant strains, 3-6 biological replicates per age group;<br>**Output:** defining of maturity genes (expression changes on days 3 & 4) and aging genes (expression changes after day 4); |
| **Studies addressing the genetic background of longevity mutants and dauers** | |
| **McElwee et al. (2003)** | 1st day adults, *daf-2(e1370)* vs. *daf2(e1370)*; *daf-16(m27)*. Pools used, 2 biological replicates, 2 technical replicates of each;<br>**Output:** putative *daf-16* targets |
| **Murphy et al. (2003)** | Young adult *daf-2* or *age-1* vs. control, N2, or *daf-2*; *daf-16*, 1-4 replicates per comparison. Pools used. Time course of sterile mutant strain treated with *daf-2*, or *daf-2 +daf-16* RNAi, or control vector, one array per condition per time point;<br>**Output:** up in *daf-2*, longevity mediators class I;<br>down in *daf-2*, longevity suppressors class II; |
| **Wang and Kim (2003)** | N2 **dauers**<br>**Output:** expression profile of dauer larvae |
| **McElwee et al. (2004)** | 1st day adults, *daf-2(e1370)* or *daf-2(m577)* vs. *daf-2; daf-16*. Pools used, 5 biological replicates per genotype;<br>**Output:** expression profile of *daf-2* mutants (up, down); |
| **Fisher and Lithgow (2006)** | 4 populations each of sterile strains containing *daf-12(rh61rh411)* or ***daf-12(rh273)***, assayed vs. mixed stage N2 reference RNA, with dye-flip for each sample;<br>**Output:** expression profile of long-lived *daf-12(rh273)* and list of genes which are down in multiple long lived worms; |
| **Shaw et al. (2007)** | **TGF-beta adults:** comparison of the dauer-constitutive mutants *daf-7(e1372)*, *daf-7(m62)*, and *daf-1(m40)* with dauer-defective mutants *daf-3(mgDf90)*, *daf-5(e1386)*, and *daf-7(e1372)*;*daf-3(mgDf90)* double mutants at the permissive temperature, 20 C, on the first day of adulthood. Because *daf-3* and *daf-5* are epistatic to *daf-7* and *daf-1*, these comparisons should identify targets that act downstream of this linear pathway;<br>**Output:** expression profile of TGFeta adults, identification of the downstream targets; |
| **Evans et al. (2008) META-ANALYSIS** | ***daf- 2*** summarized (Murphy et al., 2003 & McElwee et al., 2004);<br>**Output:** pooled expression profile of *daf-2* mutants ("broad *daf-2* up", "broad down in *daf-2"*); |
| **Studies addressing the polyphenolic action in *C. elegans*** | |
| **Viswanathan et al. (2005)** | Young adults, ***daf-16*** or **N2**, **resveratrol**-treated worms vs. untreated, pools used, 4 biological replicates per strain;<br>**Output:** expression profile of N2 and *daf-16* treated with resveratrol**;** |
| **Studies addressing the immune response of *C. elegans*** | |
| **Troemel et al. (2006)** | *fer-15(b26)*ts;*fem-1(hc17)*; wildtype; PA14, or gacA mutant PA14;<br>**Output:** expression profile of *Pseudomonas aeruginosa* infected nematodes 4 h and 8h after infection; |
| | *daf-2* vs *daf-2*;***pmk-1***;<br>**Output:** distinguishing targets either of PMK-1 (p38 MAPK) or DAF-16; |
| **Shapira et al. (2006)** | *P. aeruginosa* Infection;<br>**Output:** expression profile of *P. aeruginosa* infected nematodes; |
| **Evans et al. (2008) META-ANALYSIS** | ***P. aeruginosa* infection** summarized (Shapira et al. 2006 & Troemel et al. 2006);<br>**Output:** pooled expression profile of *P. aeruginosa* infected nematodes; |

**Overrepresented gene expression mountains according to Kim et al. (2001)**

Multiple components of the same intracellular regulatory pathway frequently display tight spatio-temporal coregulation. Such organization in synexpression groups is thought to optimize the transcriptional control over regulatory pathways (Niehrs and Pollet, 1999; Zaslaver et al., 2004). Kim et al. (2001) assembled data from 553 *C. elegans* DNA-microarray experiments, including many growth conditions, mutants and developmental stages. From this, they compiled a three-dimensional gene expression map, where highly co-regulated genes group together in terrain map mountains (also called mounts). Some of the mounts cluster genes together that are expressed in similar tissues, whereas other mountains group genes that have similar cellular functions. Hence, 30 mountains could be assigned to a potential physiological importance, by showing the enrichment of particular sets of genes. Table 19 displays all gene expression mountains, in which at least DEGs in either $Q_{longevity}$ or $Q_{all}$ display overrepresentation (additional information about all 44 mountains, exact numbers of up- and down- regulated genes, RFs and statistics can be found in Supplementary Table 9).

In a first comparison of the pooled groups $Q_{longevity}$ and $Q_{all}$ the differences between the longevity promoting concentrations and the pre-lifespan extending dose have been determined (Table 19, top, compare light and dark grey boxes). Emanating that some modes of Q action can be found over the whole concentration range, but that they are not the mediators of Q provoked longevity, this comparison defines overrepresented gene expression mountains which are exclusively regulated in $Q_{longevity}$. Gene expression structures, which are involved in lifespan prolongation, are suggested to be contained in these exclusively overrepresented gene expression mountains from $Q_{longevity}$. Obviously the overrepresentation of down-regulated genes in mounts 5 (no specific gene-groups assigned), 12 (no specific gene-groups assigned), 19 (enriched for genes from amino acid and lipid metabolism and CYPs), 22 (enriched for collagen) and 36 (enriched for heat shock genes), as well as up-regulated genes in mounts 6 (enriched for neuronal genes), 15 (no specific gene-groups assigned), 19 (see above) and 21 (enriched for lipid metabolism genes) distinguishes the long-living concentrations, from the pre-lifespan extending one. Other gene expression patterns are shared throughout the whole concentration range: overrepresentation of up-regulated genes in mounts 4 (sperm-enriched genes, protein kinases and phosphatases, MSPs), 8 (enriched for intestine-, protease-, carboxylesterase-, lipase-genes, antibacterial proteins and UGTs), 16 (enriched for muscle and collagen genes), 17 (collagen enriched), 32 (enriched for nucleosomal histone genes) and 35 (collagen enriched), and down-regulation of genes in mounts 1 (enriched for muscle, neuronal and PDZ genes), 14 (enriched for collagen) and 27 (enriched for genes assigned to Amino acid metabolism , energy generation).

Secondly, to see parallels to longevity mutants, dauers, immune challenged and resveratrol treated nematodes, previously published datasets were evaluated regarding overrepresented gene expression mountains. For better clarity, Table 19 shows just RF values of mountains which are overrepresented in at least $Q_{all}$ and $Q_{longevity}$; the full analysis of literature datasets for all gene expression mountains can be found in Supplementary Table 10. Outstanding is a big overlap between $Q_{longevity}$ on one side, with either *daf-2* (Evans et al., 2008) or TGF-beta mutants (Shaw et al., 2007); $Q_{longevity}$ shares several longevity attendant prominent mountains, as well as the direction of regulation with these mutants. These findings confirm results from GO and KEGG pathway analysis, but also from *daf-2* mutant lifespan assays.

Strikingly, mountain 4 – overrepresented in Q treated nematodes throughout the whole concentration range – is also overrepresented in *daf-12(rh273)* (Fisher and Lithgow, 2006) what points towards partly similar gene expression patterns (additional common overrepresented mounts

underpin this assumption). Interestingly resveratrol treated *daf-16* mutants (Viswanathan et al., 2005) display also overrepresentation in mountain 4, whereas their wildtype counterparts do not display this pattern. This result suggests that genes in mountain 4 are regulated in part by the absence of DAF-16 (because when *daf-16* is missing, they are expressed). In general these findings show that the absence of DAF-12 or DAF-16 induces expression of genes in mountain 4. Presumbly both palyers act in the same pathway to mediate this response. The results in Q treated nematodes suggest that Q interferes with this pathway.

An overrepresentation of mount 1 in Q treated nematodes resembles a finding from dauers (Wang and Kim, 2003). The comparison to resveratrol treated N2 (Viswanathan et al., 2005), reveals only similarities in the overrepresentation of down-regulated genes in mountain 14 (enriched for collagen), whereas resveratrol treated *daf-16* mutants show higher similarities to Q treated worms (overrepresentation of up-regulated DEGs in mountains 4, 16, 17 and down-regulated DEGs in 1, 14, 17). Noteworthy is the big difference in the regulated mountains in resveratrol treated *daf-16* and N2 nematodes, which suggests that DAF-16 plays an inhibitory role for resveratrol-induced gene expression. Since resveratrol treated *daf-16* mutants and Q treated nematodes share a notable amount of overrepresented mounts suggests that DAF-16 is suppressed due to Q treatment.

The comparison to gene expression mountains regulated by PMK-1 (Troemel et al., 2006) reveals several similarities: all by PMK-1 regulated mounts are also regulated by $Q_{longevity}$. Regulation in mountains 12, 15, 19 and 21 display even the same direction of regulation. This suggests, that Q-induced transcriptional responses are, in parts, mediated by innate immunity pathways.

No analogy can be assigned to overrepresentation in mount 35. All of these findings give first hints, that not just one genetic pathway mediates Q action *in vivo*, but rather the complex interplay of genetic structures, partly taken from classic genetic pathways, as ILS and TGF-beta, dauer related structures (which itself combines numerous pathways, as TGF-beta and ILS) and also immune-responsive patterns. A more detailed comparison of DEGs in these various conditions (see below) will further concretize these results.

Taken together, this comparison reveals that the lifespan prolonging transcriptional characteristics of Q are included in mountains 5, 6, 12, 19, 21, 22 and 36. Interestingly, the very same gene expression mountains are also employed by various long-living mutants, suggesting a molecular basis set common to all longevity-promoting perturbations.

**Table 19 Overrepresented gene expression mounts** according to Kim et al. (2001). Shown are solely representation factors (RF) from overrepresented mounts (RF > 1) in each condition with statistical significance (*p < 0.05, **p < 0.005, ***p < 0.001). Listed are all mounts in which at least DEGs in $Q_{longevity}$ or $Q_{all}$ display overrepresentation. Additional information about RFs in all mountains from all conditions can be found in Supplementary Table 9 (for Q conditions) and Supplementary Table 10 (for references of datasets see Table 18). Bright grey fields mark mountains, which are oberrepresented in $Q_{longevity}$ compared to Qall. Red RFs mark similar regulation in $Q_{longevity}$ compared to the literature.

| mountains | | 1 | 4 | 5 | 6 | 8 | 12 | 14 | 15 | 16 | 17 | 19 | 21 | 22 | 27 | 32 | 35 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Present study** | | | | | | | | | | | | | | | | | | |
| $Q_{longevity}$ | up | | 4,87 *** | | 1,40 * | 3,62 *** | | | 4,86 *** | 3,41 *** | 2,34 ** | 1,99 * | 2,44 * | | | 8,06 ** | 17,60 *** | |
| | down | 2,19 *** | | 1,11 * | | | 6,17 *** | 4,19 *** | | | | 3,39 *** | | 2,32 *** | 5,57 *** | | | 5,95 * |
| $Q_{all}$ | up | | 7,31 *** | | | 2,84 *** | | | | 5,19 *** | 3,25 ** | | | | | 15,56 *** | 30,18 *** | |
| | down | 2,12 ** | | | | | | 5,00 *** | | | | | | | 6,14 * | | | |
| **Overrepresented mountains in the literature-datasets** | | | | | | | | | | | | | | | | | | |
| ***daf-12(rh273)*** | up | | 14,36 *** | | | | | | | | | | | | | | | |
| | down | | | | | 4,18 *** | | | | | | **23,25 ***** | | **3,94 *** | 7,29 * | | | |
| ***daf-2*** | up | | | | **2,63 ***** | 3,91 *** | | | **7,43 ***** | 1,42 * | 4,06 *** | | **2,62 ***** | 2,78 *** | | | | |
| | down | | | **1,25 *** | | 2,54 *** | | | | | | **10,43 ***** | 5,19 *** | **3,87 ***** | 8,06 *** | | | |
| **TGF-beta adults** | up | | | | **2,40 ***** | 3,44 *** | | 2,43 *** | **1,69 ***** | 6,40 *** | 2,29 *** | **2,98 ***** | **2,20 ***** | 3,22 *** | 6,63 *** | | | |
| | down | | | **2,47 ***** | | | **1,55 *** | | | | | | | | | 2,68 ** | | |
| **dauers** | all | 1,71 *** | | | **2,35 ***** | 2,12 *** | | 7,94 *** | **3,29 ***** | 5,26 *** | | **4,86 ***** | **4,92 ***** | | 6,34 *** | | | |
| ***P. aeruginosa* infection** | up | | | | **1,56 *** | 5,09 *** | | | **6,14 ***** | | | **12,85 ***** | **5,78 ***** | | 4,85 *** | 5,61 * | | |
| | down | | | | | 4,58 *** | | 2,63 ** | | 7,31 *** | | **10,68 ***** | 2,88 * | **5,00 ***** | 4,11 * | | | |
| ***pmk-1*** | up | | | | | 2,54 * | | | **3,77 *** | | | **22,01 ***** | **6,25 ** | 4,52 * | | | | |
| | down | | | | | 4,07 ** | **9,46 *** | | | | | | | | | | | |
| **N2 + resveratrol** | up | | | | | | | | | | | | | 7,16 *** | | | | |
| | down | | | | | | | 3,40 *** | | | | | | | | | | |
| ***daf-16* + resveratrol** | up | | 3,43 *** | | | | | 4,48 *** | | 5,86 *** | 2,31 *** | | | 7,26 *** | | | | 8,53 *** |
| | down | 1,28 *** | | | 1,47 *** | | | 1,49 *** | 1,30 * | | 1,49 * | 1,50 * | 1,50 * | | | | | |
| **Description gene expression mountains according to Kim et al. (2001)** | | Muscle, neuronal, PDZ genes | Sperm-enriched genes, protein kinases, proteinphosphatases, major sperm proteins | | Neuronal genes | Intestine, UGT protease, carboxylesterase, lipases, antibacterial proteins | | Collagen | | Muscle, collagen | Collagen | Amino acid metabolism, lipid metabolism, cytochrome P450 | Lipid metabolism | Collagen | Amino acid metabolism, energy generation | Nucleosomal histones | Collagen | Heat shock |

## Overlapping DEGs in $Q_{longevity}$ and selected datasets

In the next step the numbers of overlapping DEGs from the datasets in the literature and Q treated nematodes were analyzed and significance of overlaps were defined for the same direction of regulation, as well as contrary regulation. Goal of this approach is to further constrain molecular mechanisms underlying the longevity promoting gene expression pattern in response to $Q_{longevity}$ treatment (results for single concentrations as well as $Q_{all}$, and comparison to all 13 DNA-microarray studies can be found in Supplementary Table 11). Table 20 summarizes the number of overlapping DEGs, and the respective RF- and p-values between $Q_{longevity}$ and the other conditions. All conditions - except resveratrol treated wild type nematodes – display significant overlaps with Q, a finding that further supports the conclusion drawn from gene expression mountain analysis. Therefore it is suggested, that major longevity assuring mechanisms are shared in $Q_{longevity}$ and long-living mutants (*daf-12*, *daf-2* and TGF-beta adults), as well as dauers, and that these mechanisms also include immune responsive elements. Furthermore it can be concluded, that resveratrol- and Q-mediated longevity in wild type nematodes, are based on differing mechanisms. However, resveratrol treated *daf-16* nematodes, which are also long-lived compared to untreated *daf-16* controls, share DEGs with Q, suggesting that DAF-16 suppression is a consequence of Q treatment. However, since similar patterns have been assigned also to Q 50 µM, it is questionable whether these DAF-16 dependent mechanisms provoke the longevity effect.

**Table 20 Overview of overlapping DEGs, according RF- and p-values between $Q_{longevity}$ and respective other conditions** (datasets from the literature, listed in Table 18).

| | | $Q_{longevity}$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | up (620) | | | down (1261) | | |
| | | genes | RF | p | genes | RF | p |
| ***daf-12(rh273)*** | **up (69)** | 38 | 17,77 | 0,00E+00 | 3 | 0,69 | 1,79E-01 |
| | **down (125)** | 8 | 2,06 | 2,50E-02 | 17 | 2,16 | 1,35E-03 |
| ***daf-2*** | **"broad up" (1155)** | 98 | 2,74 | 0,00E+00 | 76 | 1,04 | 4,51E-02 |
| | **"broad down" (905)** | 37 | 1,32 | 1,68E-02 | 131 | 2,30 | 0,00E+00 |
| **TGF-beta adults** | **up (1997)** | 132 | 2,13 | 0,00E+00 | 209 | 1,66 | 2,16E-14 |
| | **down (3728)** | 35 | 0,30 | 0,00E+00 | 86 | 0,37 | 0,00E+00 |
| **dauers** | **up/down (1663)** | 89 | 1,73 | 1,18E-07 | 211 | 2,01 | 0,00E+00 |
| ***P. aeruginosa* infection** | **up (446)** | 44 | 3,18 | 9,75E-12 | 86 | 3,06 | 0,00E+00 |
| | **down (263)** | 40 | 4,91 | 0,00E+00 | 26 | 1,57 | 6,84E-03 |
| ***pmk-1*** | **up (97)** | 7 | 2,33 | 2,06E-02 | 23 | 3,76 | 2,00E-08 |
| | **down (47)** | 2 | 1,37 | 2,52E-01 | 3 | 1,01 | 2,32E-01 |
| **N2 + resveratrol** | **up (118)** | 4 | 1,09 | 1,96E-01 | 2 | 0,27 | 1,42E-02 |
| | **down (196)** | 2 | 0,33 | 4,03E-02 | 14 | 1,13 | 9,88E-02 |
| ***daf-16* + resveratrol** | **up (1028)** | 123 | 3,86 | 0,00E+00 | 55 | 0,85 | 2,35E-02 |
| | **down (2235)** | 51 | 0,74 | 2,80E-03 | 166 | 1,18 | 2,66E-03 |

To gain deeper functional insights into aging-related genes, the significantly overlapping DEGs of $Q_{longevity}$ samples and respective datasets were examined further with a GO- and InterPro-cluster analysis (Fig. 29, Supplementary Table 12). InterPro is a database which categorizes proteins in protein families, domains and functional sites. The GO- and InterPro-cluster analysis groups GO- and InterPro- terms together which are based on the same genes. Thus, it assigns a minimal number of biological functions and pathways to each group of overlapping DEG. Figure 29 depicts the relationship of transcriptional profiles either in $Q_{longevity}$ to the other conditions and shows the results of the cluster analysis; for clarity, only the most significant proxy per

GO/InterPro- cluster is shown. Overlaps with datasets for dauers and resveratrol treated wild type have been excluded in this cluster analysis, since the direction of regulation for dauers could not be extracted from the literature and since resveratrol treated wild type do not significantly overlap with $Q_{longevity}$.

**Comparing to studies addressing the genetic background of longevity mutants**

*Comparing to* daf-12(rh273)*:* The RFs for overlapping genes (both up- and down-regulated) of $Q_{longevity}$ and *daf-12(rh273)* are significantly enhanced (Fig. 29 A), suggesting that longevity effected by *daf-12(rh273)* as well as $Q_{longevity}$ may share similar transcriptional mechanisms. Interestingly, the commonly down-regulated section revealed eight DEGs (*bre-1*, *clec-4*, *clec-66*, *dod-22*, F35E12.5, F55G11.5, M02F4.7, *pho-1*), which were found also repressed in *daf-2* and proposed to be down regulated in multiple longevity backgrounds (Fisher and Lithgow, 2006). The cluster analysis revealed one cluster in each significant intersection group (major sperm protein for commonly up-regulated genes and C-type lectin-like for commonly down-regulated genes). On the other hand, it is noteworthy that there also exists a significant group of eight genes, which were up-regulated in $Q_{longevity}$ but down-regulated in *daf-12(rh273)* mutants. These contrary regulated genes are coding for CUB-like region proteins.

Noteworthy at this point is that the locus *daf-12* is very complex. DAF-12 is a nuclear hormone receptor that is required for regulating multiple stage-specific aspects of nematode development (Antebi et al., 2000; Snow and Larsen, 2000). It encods multiple alternatively spliced isoforms and thereby is capable of mutating to daf-d, daf-c and heterochronic phenotypes independently (Savage-Dunn, 2005). In the mutant lifespan assay performed in this study the *daf-12* strain DR20 have been used, which carrys the mutation in the allele m20. This strain is dauer-defective (daf-d) and short-lived (Larsen et al., 1995; Riddle et al., 1981). The DNA-microarray dataset used here have been evolved from the comparison of daf-d *daf-12(rh61rh411)* and daf-c *daf-12(rh273)*. Therefore the conclusion drawn from mutant lifespan assays (namely: *daf-12(m20)* is not required for Q 200 µM provoked longevity, since mutants still had an extended mean lifespan) and the comparison of *daf-12(rh273)* evolved datasets to $Q_{longevity}$ provoked transcriptional output, have not compulsory be equal and thus, can divergate.

*Comparing to* daf-2*:* $Q_{longevity}$ shares a clear significant amount of down-, up- and a slightly significant amount of contrary (up in $Q_{longevity}$/down in *daf-2*) regulated DEGs compared to *daf-2* mutants (Fig. 29 B). The GO- and InterPro- cluster analysis of the intersection of up-regulated genes revealed five statistically significant clusters, such as determination of adult lifespan, lipid metabolic process and oxidoreductase activity (Supplementary Table 12). From the intersection with down-regulated transcripts emerge four clusters, as again determination of adult lifespan and UDP-glycosyltrasferase activity. These results suggest that the lifespan enhancement in $Q_{longevity}$ arises, in parts, from similar mechanisms like in *daf-2* mutants, indicated by an induction of the biotransformation system, changes in the metabolic status and up-regulation of longevity mediators as well as down regulation of longevity suppressors (cf Murphy et al., 2003; Supplementary Table 11). Beside these similar modes of action, however, also other mechanisms exist (e.g. *daf-2*-contrarian), which may contribute to life extension as well.

*Comparing to TGF-beta mutant adults:* The overlap between the gene lists for $Q_{longevity}$ and TGF-beta mutant adults is significant both in the up- and the contrary regulated section ($Q_{longevity}$ down/TGF-beta mutants up) (Fig. 29 C). The cluster analysis from the up-regulated section reveals e.g. the terms catalytic activity and active transmembrane transporter activity, and from the opponent regulated intersection e.g. peptidase 28 and carbohydrate metabolic process

(Supplementary Table 12). These results suggest that $Q_{longevity}$ may share up-regulation of particular genetic structures with TGF-beta mutants but also the inverse genetic mechanism (repression of structures, which were up-regulated in TGF-beta). 45 genes out of the contrary intersection are also down in *daf-2*, what hints towards an overlap of TGF-beta and ILS signaling, with opposing regulation of the same genes.

**Comparison to studies addressing the genetic background of induced immune response and *pmk-1***

*Comparing to* P. aeruginosa *infected nematodes:* Findings from mutant lifespan assays with Q exposure (see section 3.6.1) revealed an involvement of the innate immunity p38 MAPK pathway (represented by *sek-1*), suggesting that immune challenge might induce similar transcriptional profiles as Q treatment. Hence, to check this assumption, DEGs resulting from $Q_{longevity}$ treatment were matched to genetic profiles resulting from an immune response due to infection with a pathogenic bacteria (*P. aeruginosa* infection: Shapira et al., 2006; Troemel et al., 2006; summarized in Evans et al., 2008) (Fig. 29 D). Significant overrepresentation for DEGs in $Q_{longevity}$ could be noticed in all kinds of intersections (both up; up in $Q_{longevity}$/down in infection; both down; up in infection/down in $Q_{longevity}$). The cluster analyses of equally regulated sections reveal common up-regulation in e.g. lipid metabolic processes, monooxygenase activity, and genes coding for UDP-glucuronosyl/UDP-glucosyltransferase as well as a concurrent down-regulation in particular parts of catalytic activity. Contrary intersections contain genes coding for oxidoreductases and serin-peptidases ($Q_{longevity}$ up/infection down) and for structural constitutes of cuticle ($Q_{longevity}$ down/infection up), respectively.

*Comparing to PMK-1 regulated genes:* Troemel et al. (2006) identified specific immune response genes, which are regulated by PMK-1 (p38 MAPK), an immune response pathway in *C. elegans*, which presumably works in parallel or down-stream of the ILS pathway. They compared the genes determined as induced by PMK-1 to genes found regulated in *P. aeruginosa* infected nematodes and found a significant overlap. The evaluation of the overlap of Q treated nematodes with DEGs regulated by PMK-1 (Fig. 29 E) revealed significant overlap for $Q_{longevity}$ in respective commonly up-regulated and contrary sections (down-regulated by $Q_{longevity}$/up due to PMK-1). To the gene classes which are contrary regulated belong CUB-like genes, ShKtoxins, C-type lectins, lysozymes, proteases and others.

**Comparing to resveratrol treated *daf-16* mutants**

Both, the up- and down-regulated intersections, revealed significant RFs (Fig. 29 F). Four clusters arose from the commonly up-regulated overlap, including major sperm proteins and nematode cuticule collagen. The commonly down-regulated DEGs educed nine clusters, such as axon guidance, nervous system development, short-chain dehydrogenase and locomotion. The fact that just five genes out of these significant intersections of $Q_{longevity}$ and resveratrol treated *daf-16* mutants are also regulated in resveratrol treated wild type worms (Tables 22 and 23), suggests that resveratrol blocks the expression of these genes through DAF-16 in resveratrol treated wild type worms. When DAF-16 is absent, these transcripts were expressed. Viswanathan et al. (2005) suggested that resveratrol blocks *sir-2.1*, which acts under normal conditions on longevity through differing pathways: (i) activation of DAF-16 responsive genes and (ii) repression of *abu-11* and other ER-stress-response genes. Due to resveratrol's repression of *sir-2.1*, the ILS pathway is blocked, but the ER stress-response pathway becomes de-repressed and leads to lifespan extension in wild type worms. Thus, resveratrol mediated longevity is in-

dependent of the ILS, a finding that is confirmed by the life extension of *daf-16* nematodes due to resveratrol.

However, the significant overlaps of $Q_{longevity}$ and resveratrol exposed *daf-16* (in contrast to resveratrol treated wild type worms), in turn indicates that possibly DAF-16 is suppressed due to $Q_{longevity}$ (at least in certain tissues) or that at least some events caused by resveratrol in *daf-16* mutants resemble structures in $Q_{longevity}$ treated worms.

**Relationship between the various conditions and direction of regulation**

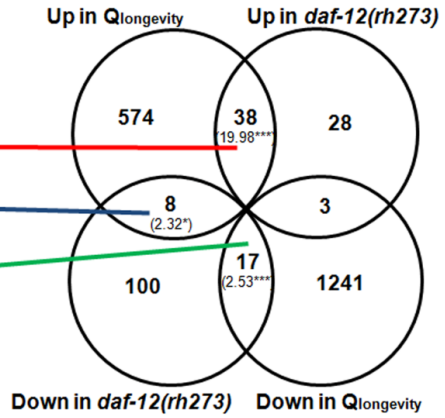
Since significant overlaps with various datasets from long-living mutants and other conditions could be shown for $Q_{longevity}$ it was interesting to ask, how the transcriptional output is composed, how the genetic players adhere and what can be learned about their co-operation. Therefore it was first necessary to understand to which extent the single conditions cohere to each other. In the first step, it will systematically be analyzed to what extent the DEGs overlap for each combination of experimental conditions (Table 21; for RF- and p-values see Supplementary Table 13). The second part of the analysis will focus on the interdependency of Q-mediated pathways, and therefore investigate simultaneous overlap between $Q_{longevity}$ and two other experimental conditions, respectively (Tables 22 and 23, additional information about RF- and p- values in Supplementary Tables 14 ($Q_{longevity}$ up) and 15 ($Q_{longevity}$ down)); this should, for example, reveal whether Q effects via *daf-2* are dependent or independent of TGF-beta-mediated effects of Q.

Table 21 reveals that a significant proportion of DEGs are shared between single conditions, both in common and contrary sections (numbers in red; see Supplementary Table 13 for additional information). This result is not surprising in light of the observation of overlapping gene expression mountains between conditions (see above). Moreover, all of the aforementioned conditions have been formerly described to display significant overlapping DEGs with at least one of the other conditions: (i) *daf-2* and TGF-beta adults (Shaw et al., 2007); (ii) *daf-2*, dauers and *daf-12(rh273)* (Fisher et al., 2006); (iii) *P. aeruginosa* infection, *pmk-1* (Troemel et al., 2006; Evans et al., 2008). To interpret and discuss the results, e.g., by considering all DEGs in the overlapping intersections, is beyond the scope of this work. Future studies should tie on this comparison, add additional datasets and thereby help to further clarify interlinks and mechanisms of genetic signaling. Just one notable aspect will be considered further in the following.

Results for *daf-2* mutants have been revealed in big parts due to the comparison of *daf-2* single mutants vs. *daf-2*; *daf-16* double mutants (Murphy et al., 2003; McElwee et al., 2004). It had been suggested that most of the obtained DEGs are DAF-16 targets. The finding that *daf-2* mutants and resveratrol treated *daf-16* mutants display a slight significant overlap in commonly up- (67 genes) and down-regulated (126 genes) DEGs (Table 20) contradicts this assumption and underlines that DAF-16 is not the sole down-stream effector of DAF-2. If this would be true, both conditions (notwithstanding of the treatement of *daf-16* with resveratrol) would not share this amount of commonly regulated DEGs. Therefore it is likely that another down-stream pathway exists, which is presumably affected by low ILS. Possibly, target genes that were so far thought to be controlled by DAF-16 are in fact (additionally or solely) regulated by other ILS effectors than DAF-16. Candidate mechanisms are yet to be identified players of the p38 MAPK pathway, a cross-regulation by TGF-beta, dependent or independent DAF-12 germline signaling but also another yet undefined pathway.

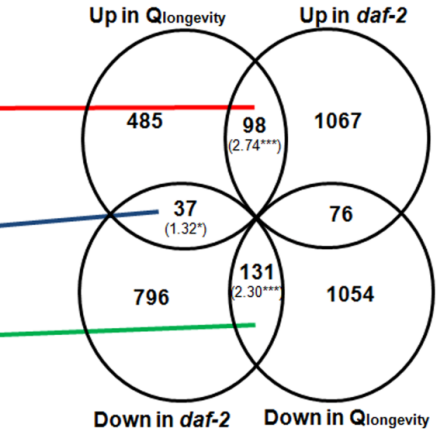
**A**

| | |
|---|---|
| IP IPR000535 | Major sperm protein (7) |
| IP IPR016186 | C-type lectin-like (3) |
| IP IPR003366 | CUB-like region (2) |



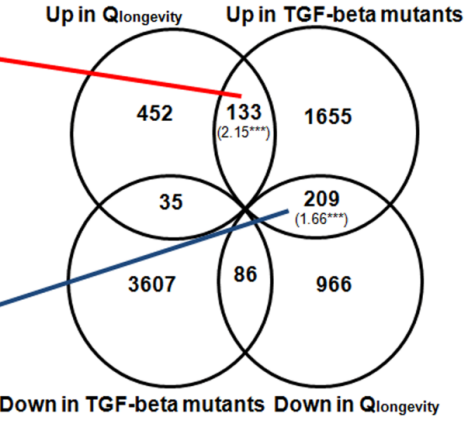

**B**

| | |
|---|---|
| IP IPR014044 | SCP-like extracellular (4) |
| MF GO:0016491 | oxidoreductase activity (13) |
| BP GO:0006629 | lipid metabolic process (6) |
| BP GO:0008340 | determination of adult life span (7) |
| BP GO:0008152 | metabolic process (24) |
| MF GO:0004497 | monooxygenase activity (3) |
| BP GO:0008340 | determination of adult life span (6) |
| IP IPR000742 | EGF-like, type 3 (4) |
| CC GO:0045111 | intermed. filament cytoskeleton (3) |
| MF GO:0008194 | UDP-glycosyltransf. activity (3) |




**C**

| | |
|---|---|
| MF GO:0042302 | structural constituent of cuticle (17) |
| CC GO:0005576 | extracellular region (8) |
| IP IPR002347 | Glucose/ribitol dehydrogenase (3) |
| CC GO:0016021 | integral to membrane (51) |
| MF GO:0003824 | catalytic activity (32) |
| MF GO:0022804 | active transmembrane transporter activity (5) |
| IP IPR013032 | EGF-like region, conserved site (9) |
| MF GO:0048037 | cofactor binding (9 ) |
| CC GO:0005882 | intermediate filament (4) |
| CC GO:0030054 | cell junction (6) |
| MF GO:0016831 | carboxy-lyase activity (3) |
| BP GO:0005975 | carbohydrate metabolic process (9) IP |
| IPR002035 | von Willebrand factor, type A (4) |
| CC GO:0005856 | cytoskeleton (10) |
| IP IPR008758 | Peptidase S28 (3) |
| MF GO:0042625 | ATPase activity, coupled to transmembrane movement of ions (4) |



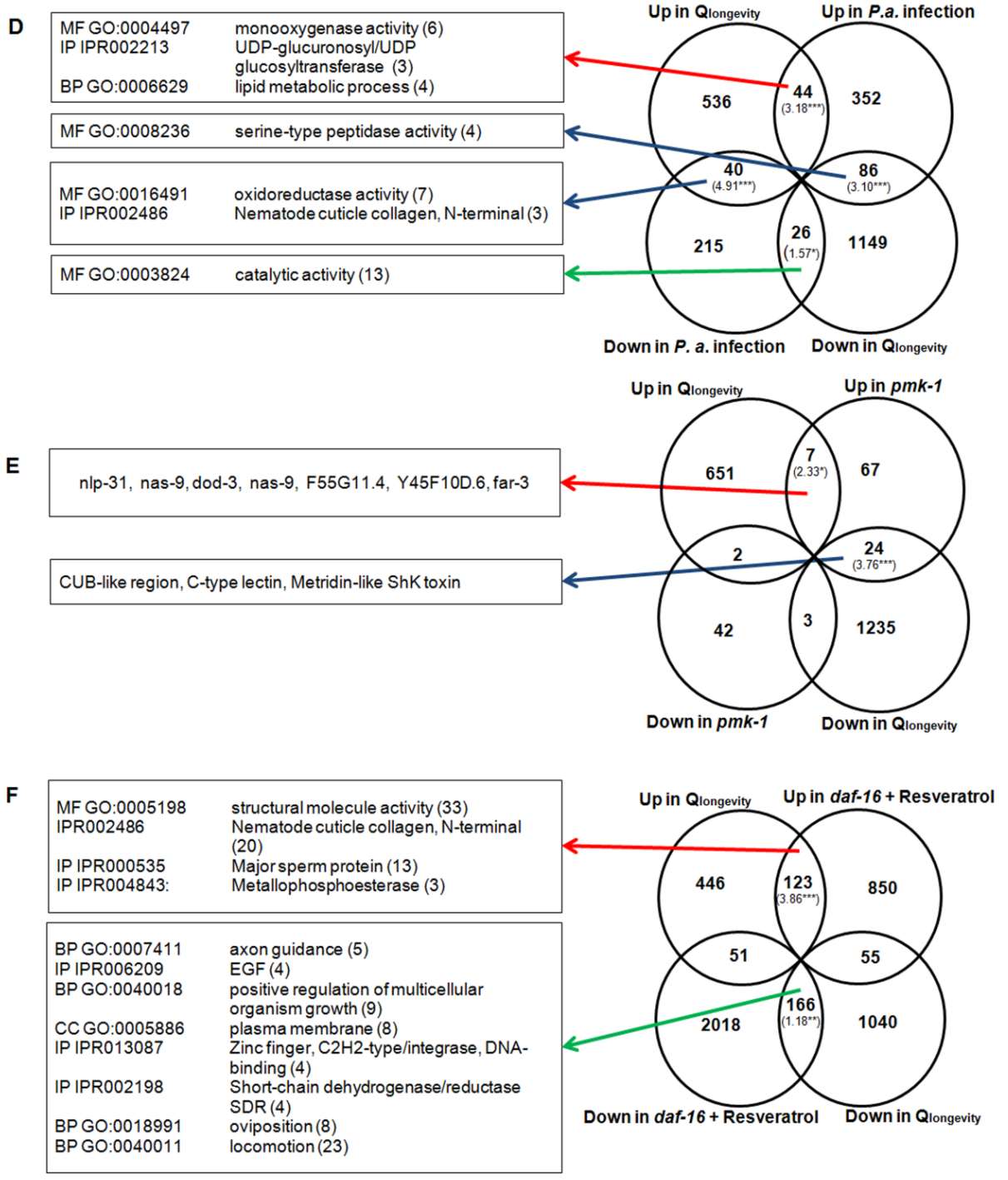



**Fig. 29 Comparison of DNA-microarray data** between $Q_{longevity}$ with **(A)** *daf12(rh273)* mutants (Fisher and Lithgow, 2006); **(B)** *daf-2* mutants (Evans et al., 2008); **(C)** TGF-beta mutant adults (Shaw et al., 2007), **(D)** with *P. aeruginosa* immune responding *C. elegans* (Evans et al., 2008), **(E)** *pmk-1* mutants (Troemel et al., 2006) and **(F)** resveratrol treated *daf-16* mutants (Viswanathan et al., 2005) using Venn diagrams. The up- and down-regulated genes in respective datasets were compared with DEGs in $Q_{longevity}$ treatment. Significant overlap is indicated by RF values in brackets (*p < 0.05, **p < 0.005, ***p < 0.001). Additional information about all RF values in single PP concentrations and with comparisons to additional publications can be found in Supplementary Table 11. The left section displays a GO- and InterPro- cluster analysis of overlapping DEGs in respective intersections, listed are the most significant term per cluster (IP: InterPro, MF: Molecular Function; BP: Biological Process; CC: Cellular Function). Gene names and p-values can be found in Supplementary Table 12. Red arrows indicate an origin in a commonly up-regulated intersection, green arrows originate in a commonly down-regulated intersection. (Note: no clusters were found in the comparison with *pmk-1* mutants, due to the small number of DEGs; instead single terms are shown).

In the next step, the interdependency of Q-mediated pathways have been analyzed, and the number of overlapping DEGs of either $Q_{longevity}$ up- or down-regulated genes with two conditions coevally have been determined (Tables 22 and 23). Although there are many interesting findings, this study will be restricted to the interpretation of two aspects:

*Commonly regulated transcripts in* daf-12(rh273)*, resveratrol treated* daf-16 *mutants and* $Q_{longevity}$*:* When comparing *daf-12(rh273)* up-regulated transcripts with data from other studies, there do not exist noteworthy overlaps, except for resveratrol exposed *daf-16* mutants (Table 20). From the 38 commonly regulated DEGs in $Q_{longevity}$ and *daf-12(rh273)*, 26 genes are also up-regulated in *daf-16* exposed to resveratrol (Table 22), suggesting a molecular pathway common to all three conditions. Notably, 25 out of these 26 genes are members of gene expression mountain 4, which contains sperm-enriched genes, protein kinases, protein phosphatases and major sperm proteins. Morover, most of the 26 genes are also induced by Tannic Acid, another lifespan extending polyphenol investigated in our laboratory, which further supports their physiological relevance (unpublished data).

Gene expression mountain 4 is expressed throughout the whole concentration range of Q treated nematodes (and also Tannic acid treated nematodes), and thus also in non-lifespan extending exposures (e.g. Q 50 µM, Tannic acid 300 µM); this suggests that the 26 overlapping genes are presumably not responsible for lifespan extension. However, they could still be involved in Q mediated action *in vivo*, even though not predominantely in life extension. Both *daf-12* and *daf-16* are important players in longevity mediated through germline signaling (reviewed in Panowski and Dillin, 2009). Since sperm-enriched- and male-enriched genes out of gene expression mountain 4 are predominantely induced, suggests that this pathway could be also important for Q's action. However, the full mechanism of germline signaling is not completely understood so far, thus, this assumption needs further clearance in the future.

*Commonly and contrary regulated genes in* daf-12(rh273)*,* daf-2*, TGF-beta adults,* P. aeruginosa *infected,* pmk-1 and $Q_{longevity}$: Owing to the huge overlaps of Q with either *daf-12(rh273)*, *daf-2*, TGF-beta, *pmk-1* or infected nematodes (Table 20), it was interesting to know whether genes common to all conditions exist and, if yes, whether they exhibit the same direction of regulation. A large fraction of DEGs from the contradictory regulated intersection of $Q_{longevity}$ and *P. aeruginosa* infected nematodes ("up in infection/down in $Q_{longevity}$", 86 genes, Table 23) 'is also down-regulated in *daf-2* (37 genes) and *daf-12(rh273)* (10 genes); this again suggests overlapping molecular pathways employed by different perturbation conditions. Among the 37 commonly regulated genes ("down in *daf-2* and $Q_{longevity}$/up in *P.aeruginosa* infection") are 7 CUB-like genes, 4 C-type lectins and 3 genes coding for aging relevant genes (Fig. 29, Supplementary Table 12). Interestingly, CUB-like genes were found also activated in induced immune response pathway modulated by PMK-1 (Troemel et al., 2006). Indeed, 15 genes out of the 86 gene overlap ("up in infection/down in $Q_{longevity}$", Table 23) are also induced in *pmk-1* mutants, and belong to the CUB-like region genes, C-type lectins and Metridin-like ShK toxins. The proteins evolved from CUB-like genes could be involved in a diverse range of functions, including complement activation, developmental patterning, tissue repair, axon guidance, angiogenesis, cell signaling, fertilization, haemostasis, inflammation, neurotransmission, receptor-mediated endocytosis and tumor suppression (Abdul Ajees et al., 2006; Perry et al., 2007). Interestingly, Troemel et al. (2006) found that some genes are commonly regulated by PMK-1 and DAF-16, but with inverse regulation. Into this group fall among others the CUB-like ones (which were found down-regulated due to $Q_{longevity}$ exposure). They proposed a model where the PMK-1 pathway on one hand and the DAF-2/DAF-16 pathway on the other, regulate common and exclusive immunity genes in parallel, some with contrary direction, but all contributing to im-

munity and thereby to a prolonged lifespan. The ILS promotes longevity furthermore not just through specialized immunity genes, but also due to regulation of general stress response genes (more details can be found in the discussion section 4.6.1). Since $Q_{longevity}$ display a notably amount of down-regulated CUB-like genes (down-regulation is shared with *daf-2*, while up in *P. aeruginosa* infection and *pmk-1* mutants), it is suggested that DAF-16 represses these genes following Q treatment. On the other hand $Q_{longevity}$ shows significant overlap with up-regulated genes in *pmk-1* and therefore it is suggested, that the p38 MAPK pathway is also involved.

Considering the other converse regulated intersection of $Q_{longevity}$ and infected nematodes ("up in $Q_{longevity}$/down in infection", 40 genes), there exist also extensive overlap with up-regulated genes in TGF-beta mutants (21 genes, Table 21) and in *daf-2* (17 genes, whereof 12 together with TGF-beta). Among them are defense genes like *spp-1*, *thn-1*, *thn-2*, and biotransformations genes like *sod-3*, *sodh-1*, *gst-4* and *gst-29*, collagens and others. This proves that a shared stress response (biotransformation, other defense genes) is commonly activated by Q, TGF-beta signaling, and DAF-2.

Considering the data presented here suggests that both, the ILS (through attenuated signaling and thus de-repression/activation of DAF-16) and the p38 MAPK pathway, play important roles in Q mediated longevity, and that a complex regulation determines, which genes are up- and which are down-regulated. Probably is that the TGF-beta pathway and germline signaling (both described acting partly through DAF-12), interfere to some extent also in this regulation.

**Table 21 Overlapping genes of single datasets from the literature** (reference list see Table 18). Red numbers indicate significant overlaps (according RFs and p-values can be found in Supplementary Table 13).

| | | *daf-12(rh273)* | | *daf-2* | | TGF-beta adults | | *P. aeruginosa* infection | | dauers | N2 + resveratrol | | *daf-16* + resveratrol | | *pmk-1* | | Qlongevity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (69) | down (125) | up (1155) | down (905) | up (1997) | down (3728) | up (446) | down (263) | up/down (1663) | up (118) | down (196) | up (1028) | down (2235) | up (97) | down (47) | up (620) | down (1261) |
| *daf-12(rh273)* | up (69) | | 0 | 6 | 2 | 3 | 3 | 2 | 2 | 6 | 0 | 1 | 31 | 5 | 1 | 0 | 38 | 3 |
| | down (125) | 0 | | 13 | 34 | 31 | 17 | 30 | 9 | 32 | 0 | 0 | 10 | 14 | 15 | 2 | 8 | 17 |
| *daf-2* | up (1155) | 6 | 13 | | 4 | 324 | 61 | 59 | 63 | 207 | 8 | 12 | 67 | 172 | 9 | 3 | 98 | 76 |
| | down (905) | 2 | 34 | 4 | | 216 | 138 | 150 | 33 | 207 | 10 | 8 | 40 | 126 | 24 | 2 | 37 | 131 |
| TGF-beta adults | up (1997) | 3 | 31 | 324 | 216 | | 0 | 94 | 112 | 430 | 20 | 17 | 216 | 254 | 21 | 5 | 132 | 209 |
| | down (3728) | 3 | 17 | 61 | 138 | 0 | | 18 | 12 | 156 | 36 | 18 | 132 | 388 | 5 | 4 | 35 | 86 |
| *P. aeruginosa* infection | up (446) | 2 | 30 | 59 | 150 | 94 | 18 | | 1 | 85 | 2 | 5 | 17 | 65 | 32 | 0 | 44 | 86 |
| | down (263) | 2 | 9 | 63 | 33 | 112 | 12 | 1 | | 72 | 1 | 4 | 20 | 38 | 2 | 4 | 40 | 26 |
| dauers | up/down (1663) | 6 | 32 | 207 | 207 | 430 | 156 | 85 | 72 | | 13 | 24 | 147 | 265 | 17 | 3 | 89 | 211 |
| N2 + resveratrol | up (118) | 0 | 0 | 8 | 10 | 20 | 36 | 2 | 1 | 13 | | 0 | 42 | 3 | 0 | 0 | 4 | 2 |
| | down (196) | 1 | 0 | 12 | 8 | 17 | 18 | 5 | 4 | 24 | 0 | | 3 | 75 | 0 | 1 | 2 | 14 |
| *daf-16* + resveratrol | up (1028) | 31 | 10 | 67 | 40 | 216 | 132 | 17 | 20 | 147 | 42 | 3 | | 13 | 0 | 1 | 123 | 55 |
| | down (2235) | 5 | 14 | 172 | 126 | 254 | 388 | 65 | 38 | 265 | 3 | 75 | 13 | | 17 | 6 | 51 | 166 |
| *pmk-1* | up (97) | 1 | 15 | 9 | 24 | 21 | 5 | 32 | 2 | 17 | 0 | 0 | 0 | 17 | | 0 | 7 | 23 |
| | down (47) | 0 | 2 | 3 | 2 | 5 | 4 | 0 | 4 | 3 | 0 | 1 | 1 | 6 | 0 | | 2 | 3 |
| Qlongevity | up (620) | 38 | 8 | 98 | 37 | 132 | 35 | 44 | 40 | 89 | 4 | 2 | 123 | 51 | 7 | 2 | | 0 |
| | down (1261) | 3 | 17 | 76 | 131 | 209 | 86 | 86 | 26 | 211 | 2 | 14 | 55 | 166 | 23 | 3 | 0 | |

**Table 22 Double overlaps of up-regulated genes in $Q_{longevity}$ with two other conditions, respectively.** (References of datasets can be found in Table 18; according RFs and p-values can be found in Supplementary Table 14).

| $Q_{longevity}$ up | | *daf-12(rh273)* | | *daf-2* | | TGF-beta adults | | *P. aeruginosa* infection | | *pmk-1* | | dauers | N2 + resveratrol | | *daf-16* + resveratrol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (38) | down (8) | up (98) | down (37) | up (132) | down (35) | up (44) | down (40) | up (97) | down (47) | up/down (89) | up (4) | down (2) | up (123) | down (51) |
| *daf-12(rh273)* | up (38) | | 0 | 2 | 1 | 2 | 0 | 1 | 0 | 1 | 0 | 3 | 0 | 0 | 26 | 1 |
| | down (8) | 0 | | 1 | 3 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| *daf-2* | up (98) | 2 | 1 | | 0 | 39 | 1 | 8 | 17 | 2 | 1 | 28 | 1 | 0 | 13 | 18 |
| | down (37) | 1 | 3 | 0 | | 7 | 5 | 12 | 2 | 1 | 1 | 7 | 1 | 0 | 3 | 7 |
| TGF-beta adults | up (132) | 2 | 2 | 39 | 7 | | 0 | 7 | 21 | 2 | 1 | 41 | 2 | 0 | 26 | 20 |
| | down (35) | 0 | 0 | 1 | 5 | 0 | | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 1 | 4 |
| *P. aeruginosa* infection | up (44) | 1 | 1 | 8 | 12 | 7 | 4 | | 1 | 0 | 0 | 10 | 1 | 0 | 1 | 7 |
| | down (40) | 0 | 1 | 17 | 2 | 21 | 0 | 1 | | 0 | 2 | 7 | 0 | 1 | 5 | 4 |
| *pmk-1* | up (7) | 1 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | | 0 | 2 | 0 | 0 | 0 | 2 |
| | down (2) | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | | 1 | 0 | 0 | 1 | 0 |
| dauers | up/down (89) | 3 | 1 | 28 | 7 | 41 | 4 | 10 | 7 | 2 | 1 | | 2 | 0 | 13 | 11 |
| N2 + resveratrol | up (4) | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | | 0 | 1 | 0 |
| | down (2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 1 |
| *daf-16* + resveratrol | up (123) | 26 | 1 | 13 | 3 | 26 | 1 | 1 | 5 | 0 | 1 | 13 | 1 | 0 | | 0 |
| | down (51) | 1 | 2 | 18 | 7 | 20 | 4 | 7 | 4 | 2 | 0 | 11 | 0 | 1 | 0 | |

**Table 23 Double overlaps of down-regulated genes in $Q_{longevity}$ with two other conditions, respectively.** (References of datasets can be found in Table 18; according RFs and p-values can be found in Supplementary Table 15).

| $Q_{longevity}$ down | | *daf-12(rh273)* | | *daf-2* | | TGF-beta adults | | *P. aeruginosa* infection | | *pmk-1* | | dauers | N2 + Resveratrol | | *daf-16* + resveratrol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (3) | down (17) | up (76) | down (131) | up (209) | down (86) | up (86) | down (26) | up (23) | down (3) | up/down (211) | up (2) | down (14) | up (55) | down (166) |
| *daf-12(rh273)* | up (3) | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | down (17) | 0 | | 1 | 8 | 5 | 0 | 10 | 1 | 6 | 0 | 7 | 0 | 0 | 1 | 2 |
| *daf-2* | up (76) | 0 | 1 | | 0 | 21 | 1 | 10 | 4 | 3 | 0 | 15 | 0 | 0 | 4 | 15 |
| | down (131) | 0 | 8 | 0 | | 45 | 13 | 37 | 2 | 10 | 0 | 39 | 1 | 1 | 8 | 15 |
| TGF-beta adults | up (209) | 0 | 5 | 21 | 45 | | 0 | 28 | 12 | 8 | 1 | 78 | 0 | 1 | 16 | 26 |
| | down (86) | 0 | 0 | 1 | 13 | 0 | | 2 | 0 | 2 | 0 | 5 | 0 | 1 | 1 | 19 |
| *P. aeruginosa* infection | up (86) | 0 | 10 | 10 | 37 | 28 | 2 | | 0 | 15 | 0 | 26 | 0 | 0 | 5 | 9 |
| | down (26) | 1 | 1 | 4 | 2 | 12 | 0 | 0 | | 0 | 0 | 10 | 0 | 1 | 1 | 3 |
| *pmk-1* | up (23) | 0 | 6 | 3 | 10 | 8 | 2 | 15 | 0 | | 0 | 5 | 0 | 0 | 0 | 6 |
| | down (3) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 1 |
| dauers | up/down (211 | 1 | 7 | 15 | 39 | 78 | 5 | 26 | 10 | 5 | 0 | | 0 | 5 | 14 | 41 |
| N2 + resveratrol | up (2) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 |
| | down (14) | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 5 | 0 | | 0 | 3 |
| *daf-16* + resveratrol | up (55) | 0 | 1 | 4 | 8 | 16 | 1 | 5 | 1 | 0 | 0 | 14 | 0 | 0 | | 1 |
| | down (166) | 0 | 2 | 15 | 15 | 26 | 19 | 9 | 3 | 6 | 1 | 41 | 0 | 3 | 1 | |

**Considering interesting aging relevant genes**

Given that distinct longevity phenotypes vary to a considerable extent in underlying genetic mechanisms but share single commonly regulated genes, it is arguable that these genes may play an important role in mediating lifespan extension by master-regulating subsequent genes. In the following, therefore a list of putative aging master regulator genes will be defined based on overlaps between various array studies.

Datasets of life extended nematodes (*daf-2*, *daf-12(rh273)*, TGF-beta mutants, resveratrol treated wild type and *daf-16* nematodes) have been compiled and compared, as discussed in the previous sections. To enhance the possibility of success in extracting longevity associated genes, another dataset of polyphenol life-extended nematodes was included: Tannic acid (TA) has been shown to extend longevity in *C. elegans*, when applied in 100 µM or 200 µM (Saul et al., 2010). Transcriptome-wide microarray analysis was performed, an aging-related set of genes termed $TA_{longevity}$ (pooled results of TA 100 µM and 200 µM) was defined and then compared to $Q_{longevity}$ treated nematodes (unpublished, publication in preparation). A list of putative aging-related 39 genes was compiled based on the following criteria (Table 24): (i) common up- or down-regulation in $Q_{longevity}$ and $TA_{longevity}$ and (ii) transcriptional regulation in one or more other longevity condition.

Possibly $Q_{longevity}$ influences the life-cycle by slowering the “rate of living”, thereby causing developmental delays, and as a resulting consequence changes also life-cycle dependent gene expression. Expression variations of such genes relative to control could hence also be a consequence of a slower life, instead of the cause. To rule out these artifacts the extracted genes have been further compared to a dataset of Hill et al. (2000) who addressed the gene-expression changes during the life cycle. Specifically, it was analyzed whether the selected genes belong to the group of genes with varying expression profile during the lifecycle (Hill et al., 2000). If yes, the expression tendencies between 48 h and 60 h ($1^{st}$ day of adulthood/young adult stage due to maintaining at 25°C and therefore comparable with the stage of $Q_{longevity}$ treated worms) in Hill et al. (2000) were compared to our data. This leads to determination of genes, whose expression values are potentially modified just due to simple developmental delays. Genes with decreasing expression between 48 h and 60 h might be up-regulated in our study because of developmental delays. Contrarily, genes with increasing expression in Hill et al. (2000) would be down-regulated in our study and therefore presumably being a consequence of a possible “slower” life caused by PPs. The 11 genes which do not match these criteria of simple developmental delays are marked with an “x” in the last column of Table 24, and represent possible genetic players associated with longevity. Together with the genes, which did not alter gene expression during the life-cycle in Hill et al., the list comprises 23 interesting genes. Since many genes among them are not yet characterized and annotated, future tests will have to reveal the importance in aging in *C. elegans*, e.g. by knock-out mutants, by RNAi, or by overexpression. Several genes code for sperm-related proteins and nematode-specific peptides, suggesting that these proteins play yet unknown roles beside sperm-specific action, presumably also in longevity mediation. Below follows a short description of some of these selected genes:

C16E9.1 is a von Willebrand factor (vWF) protein. Although the majority of vWF-containing proteins are extracellular (plasma proteins like complement factors, collagens and others; Bork, 1991; Colombatti et al., 1993; Perkins et al., 1994), the most ancient ones, present in all eukaryotes, are intracellular proteins involved in transcription, DNA repair, ribosomal and membrane transport, and the proteasome. A common feature appears to be involvement in multi-protein

complexes. Proteins that incorporate vWF domains participate in numerous biological events (e.g. cell adhesion, migration, homing, pattern formation, and signal transduction), involving interaction with a large array of ligands (Colombatti et al., 1993).

*cex-1* is one of two predicted calexins in the *C. elegans* genome. Calexins, $Ca^{2+}$ binding proteins with an EF-hand motif, are involved in cellular mechanisms underlying associative learning, due to protein kinase C-dependent inactivation of voltage-dependent $K^+$-channels (Alkon et al., 1998) and on the other via interaction and activation of ryanodine receptors (Nelson et al., 1999). Furthermore, it could be shown that *cex-1* is expressed in several muscular cell types and the AVB command interneuron. Overexpression can increase the rate of learning acquisition, functions in two serotonin-mediated behavioral responses, up regulation of pharyngeal pumping, and egg laying rate (Walker, 2005).

*far-7* is a fatty acid/retinol binding protein, which is positively regulated due to starvation.

Very interesting for further analysis seems to be *cpt-6*/W01A11.5, coding for a carnitine palmitoyl transferase-1a, which is required for transport of fatty acyl-CoAs into mitochondria and therefore belongs to the conserved components of mitochondrial and peroxisomal β-oxidation pathways. The hypodermis, major sites of *C. elegans* fat storage, was determined as site of function (Srinivasan et al., 2008). The authors identified *cpt-6* as involved in serotonergic regulation of fat and feeding in *C. elegans*. *cpt-6* was one of nine metabolic genes, which, when inactivated by RNAi, partially blocked the fat-reducing effect of 5-HT and fully suppressed the increased oxygen consumption due to 5-HT and also abrogated the increased feeding rate of 5-HT-treated animals, potentially due to accumulation of acyl-CoA moieties. How this picture fits with our results, as well as the results for the resveratrol treated animal and the longevity mutants *daf-2* and TGFbeta, needs further clearance. Both Q 200 µM (Pietsch et al., 2011), as well as TA 100 µM (Saul et al., unpublished) treated nematodes displayed significant decreased levels of fat content, and in Q 200 µM the pharyngeal pumping/feeding is significantly increased, while expression of *cpt-6* is decreased. Presumably there are complex interactions.

F35E12.5 is presumably responsible for a swelling response after infection (O'Rourke et al., 2006). It is a member of the complement C1r/C1s, sea urchin epidermal growth factor-related protein, bone morphogenetic protein 1 (CUB) family of proteins (Bork and Beckmann 1993), with a DUF141 motif (O'Rourke et al., 2006). CUB-like proteins are involved in many diverse functions, including complement activation, inflammation, cell signaling, and many others. This specific CUB-like transcript has yet no known orthologs in higher organisms, and their exact function in C. *elegans* is not known. F35E12.5 was identified as p38MAPK/PMK-1 regulated gene (Troemel et al., 2006) and is thought to promote immunity.

*sur-5* encodes a protein with high similarity to *H. sapiens* Acetoacetyl-coenzyme A synthetase and is involved in Ras signaling, which is required for organismal viability and many other developmental processes Because of the widespread roles of Ras signaling during development, mutations affecting the Ras pathway can cause many different pleiotropic defects.

**Table 24 Genes which are regulated in diverse longevity scenarios identified by meta-analysis.** Genes, identified as life-cycle changing genes (Hill et al., 2000), were further analyzed if expression increased (↑) or decreased (↓) between 48 and 60 h of development, to see if changes due to PP treatment could be simply a consequence of a slower developmentally rate. Genes, whose expression changes in PP treatments cannot be explained by simple life-cycle effects, are marked with an "x" in the last row. Bright grey fields indicate induction; dark grey fields indicate repression of the gene in respective condition. "*" highlights where respective gene was found regulated.

| Gene Symbol | Study / Description | Life-cycle changing DEGs | Tendency of gene expression (48-60h) | Not explainable due to developmental delays | dauer | N2 + resveratrol | *daf-16* + resveratrol | TGF-beta adults | *daf-12 (rh273)* | *daf-2* | $TA_{longevity}$ (unpublished) | $Q_{longevity}$ (present study) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C16E9.1 | Von Willebrand factor | | | | | | | | | | | |
| C33F10.1 | | | | | | | | | | | | |
| C45B2.1 | | * | ↑ | x | | | | | | | | |
| C45G9.12 | | * | ↓ | | | | | | | | | |
| *cex-1* | CalEXcitin | | | | * | | | | | | | |
| *col-118* | | * | ↓ | | | | | | | | | |
| *col-139* | Collagens (type IV and type XIII) | * | ↓ | | | | | | | | | |
| *cpt-6* | Carnitine O-acyltransferase CPTI | * | ↓ | x | | | | | | | | |
| F20A1.10 | Uncharacterized protein | * | ↑ | x | * | | | | | | | |
| F35E12.5 | CUB-like gene | * | ↓ | x | | | | | | | | |
| F58A6.9 | major sperm protein | * | ↓ | | | | | | | | | |
| *far-7* | Fatty Acid/Retinol binding protein | | | | | | | | | | | |
| *lips-17* | R07G3.2, Triacylglycerol lipase | | | | | | | | | | | |
| *msp-10* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *msp-142* | Major Sperm Protein | * | ↓ | | | | | | | | | |
| *msp-31* | Major Sperm Protein | * | ↓ | | | | | | | | | |
| *msp-38* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *msp-49* | Major Sperm Protein | * | ↓ | | | | | | | | | |
| *msp-50* | Major Sperm Protein | * | ↓ | | | | | | | | | |
| *msp-51* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *msp-56* | Major Sperm Protein | | | | | | | | | | | |
| *msp-59* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *msp-76* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *msp-78* | Major Sperm Protein | * | ↑ | x | | | | | | | | |
| *nspa-1* | Nematode Specific Peptide family/group A | | | | | | | | | | | |
| *nspd-2* | Nematode Specific Peptide family, group D | * | ↓ | | | | | | | | | |
| *nspd-5* | Nematode Specific Peptide family, group D | * | ↓ | | | | | | | | | |
| *snf-9* | | | | | | | | | | | | |
| *sodh-1* | SOrbitol DeHydrogenase family, Alcohol dehydrogenase, class V | * | ↓ | | | | | | | | | |
| *srr-4* | Serpentine Receptor, class R | * | ↓ | | | | | | | | | |
| *ssp-11* | Sperm-Specific family, class P | | | | | | | | | | | |
| *ssp-19* | Sperm-Specific family, class P | | | | | | | | | | | |
| *sss-2* | Sperm-Specific family, class S | * | ↓ | | | | | | | | | |
| *sur-5* | SUppressor of activated let-60 Ras, Acyl-CoA synthetase | * | ↓ | x | * | | | | | | | |
| T26C5.4 | | * | ↓ | | | | | | | | | |
| *tag-32* | Temporarily Assigned Gene name, S-adenosylmethionine synthetase | * | ↓ | | | | | | | | | |
| Y59E9AL.3 | | | | | | | | | | | | |
| ZK484.5 | | * | ↓ | | | | | | | | | |
| ZK596.1 | Predicted alpha-helical protein | | | | | | | | | | | |

# 4 Discussion

PPs are a potent substance class of phytochemicals, and some are capable of significantly extending the lifespan of *C. elegans* (examples are summarized in Supplementary Table 16; taken from Saul et al., 2009). They are able to induce a vast array of phenotypic responses, thus further in-depth investigations are required to rule out artifacts associated with respective test-designs and to decipher the exact underlying mechanisms that drive the observed effects. The applicability of using the model organism, *C. elegans,* to identify *in vivo* activities of natural compounds has certainly evolved and has become more differentiated (Gruber et al., 2009; Pun et al. 2010). This study aims to further our understanding regarding Q, CA, and RA mediated longevity and readdresses aforementioned challenges by answering the following questions: Does the observed longevity arise from hormesis? Are *in vivo* antioxidative properties responsible for causing the life extension? Do these PPs cause direct or indirect CR effects? Do antibacterial effects against the *E. coli* feeding strain lead to artifacts? Does the treatment with PPs cause physiological reallocations, which in turn provoke the prolonged lifespan in a disposable soma-like manner? Which genetic players and pathways are involved in longevity mediation respectively?

## 4.1 Does the test design produce artifacts?

*Antimicrobial properties:* Direct longevity effects can be discriminated from indirect antibacterial effects by feeding *C. elegans* with heat- or UV-killed OP50 (Patridge and Gems, 2007; Gruber et al., 2009). This study was able to prove that the antibacterial properties of Q and RA are independent entities and do, (if at all) influence their positive effect on lifespan extension in an ancillary range. An antimicrobial effect for CA could not be determined. Since RA administered with heat killed bacteria caused a more significant life extension compared to nematodes fed with living OP50, we can not exclude the possibility, that through *E. coli* metabolically altered and retired RA could also cause partly negative effects on *C. elegans*. The production of deleterious metabolites by proliferating bacteria can provoke harmful effects on *C. elegans* (Gems and Riddle, 2000; Garigan et al., 2002). Hence, it has been suggested that nematodes gain health benefits if the bacterial food source (the gram-negative *E. coli* OP50 feeding strain) is debilitated by antimicrobial substances.

*Transgenerational effects of PPs:* Furthermore it was possible to exemplarily demonstrate for Q that transgenerational effects, which could provoke adaptive or additive effects on lifespan due to variations in differing exposure times, are negligible and therefore do not cause artifacts in the choosen test design. Since lifespan assays with whole-life-time Q-treated F1 nematodes revealed slightly higher statistical robustness than L4-onwards treated P0 nematodes, it was deemed adequate to choose the F1 scenario as standard exposure regime for all following experiments.

## 4.2 Is longevity promoted via hormesis?

For all three PPs, the lifespan assays show a biphasic, inverted U-shaped concentration-effect relationship, similar to a classical hormetic dose response curve and therefore could be classified as hormetins (Rattan, 2008). The beneficial effects of mild stress on aging and longevity have been studied for several years (for review see Le Bourg, 2009), and this study addresses this aspect in fine detail. An important event in hormetical responses is the induction of one or

more stress response pathways, such as the stress responsive heat shock protein (HSP) pathway(s) (Rattan, 2008). Increased levels of HSP can provide several benefits against crucial aspects of aging, including the protection against molecular damage. This study found that the expression of various HSPs is enhanced by Q and RA, suggesting that HSP induction may play an important role for the hormetic action of these PPs. In contrast, the HSP response was less pronounced for CA; however, it is important to note that the analysis was restricted to specific *hsp*-genes and other transcripts may have returned other results. In classical hormesis, stress response pathways are directly induced by hormetins. Alternatively, *hsp*-induction by Q and RA may be due to an indirect bactericidal effect on the OP50 feeding strain. Therefore, inhibition of bacterial growth could elicit the production of deleterious metabolites, which in turn would induce the expression of *hsp*-genes. In this scenario, the observed effects would not be a direct hormetic response, but at most an indicator of indirect hormesis, or possibly an artifact of the experimental design. Addressing this question, for example via the use of heat- or UV-killed OP50, is clearly of importance as it will help to standardize handling procedures (Patridge and Gems, 2007; Gruber et al., 2009) and further define the suitability of *C. elegans* as a model organism for testing pharmacological compounds.

## 4.3 Do direct or indirect CR effects contribute to life extension?

The speed of pharynx contractions was determined to investigate whether PPs reduce food uptake and thereby impose a CR response; such an effect is thought to underlie longevity in slow-pumping *eat-2* mutant (Lakowski and Hekimi, 1998). In accordance with previous reports, pharynx contractions declined gradually with increasing age (Huang et al., 2004). However, all three PPs resulted in an increased pumping rate at all three time points, thus excluding a CR response due to reduced food uptake. Indeed, the enhanced food intake observed in treated nematodes could indicate an increased availability of energy for respective nematodes. Unforced to prioritize, more energy could be distributed to various life processes, thus PPs treated nematodes would have advantages, without drastic disadvantages in major fields.

Furthermore, another source of possible CR was investigated, namely the avoidance of food via a PPs based gustative distraction. Nematodes possess amphid and phasmid organs to distinguish between diverse odors and tastes, detect food, avoid toxic substances and find possible mating partners. Since none of the PP supplemented foods caused a significant repulsion, as tested by the attraction assay, the hypothesis of direct CR was rejected. The moderate (but insignificant) tendency towards an avoidance of Q and RA supplemented bacterial spots could be correlated to the negative impact on the growth rate of *E. coli*, or due to modifications of the PPs by bacterial metabolism. This should be addressed in future tests.

Having excluded PP induced alterations in food uptake, it was essential to investigate whether metabolic change by means of a *sir-2.1* operation is affected by PPs. Indeed, CA and RA did not significantly prolong lifespan in *sir-2.1* mutants which may be characterized by an intrinsic CR response (Tissenbaum and Guarente, 2001; Wood et al., 2004). Thus, it could be possible that both PPs activate an indirect CR response in the nematodes (for detailed discussion see section 4.6.2). Further experiments are called for in the future, to test this hypothesis in clearer detail.

## 4.4 Does disposable soma-like re-allocation of resources take place?

According to the disposable soma theory (Kirkwood, 1977, 1988), the total amount of energy available to an organism is divided into maintenance, growth and reproduction. Therefore, it was analyzed whether an extension of lifespan by PPs result in trade-offs within other parts of physiology. Experimental support for the disposable some theory was obtained in mice and *Drosophila melanogaster*, where a reduction in body size correlates with longevity (Flurkey et al., 2001; Nielsen et al., 2008). Such trade-offs have also been observed in *C. elegans*, where Harrington and Harley (1988) noted a reduction of offspring in response to Vitamin E mediated longevity. Similarly, Saul et al. (2009, 2010) discovered that Catechin and Tannic acid mediated longevity was associated with an attenuation of growth. These findings encouraged the study of several fitness parameters to verify whether physiological trade-offs occur in *C. elegans.*

First, PPs induce a delay in the onset of reproduction (though statistically significant only in RA exposed nematodes). Szewczyk et al. (2006) compared *C. elegans* grown on NGM with worms maintained on chemically defined minimal liquid medium (CeMM - *C. elegans* Maintenance Medium) and observed that CeMM induced longevity and prolonged development and the reproductive period, which implicates that longevity can be the result of slower rate of living. This could also be the case with PP treatment.

Second, the growth of exposed and control worms was compared. PPs induce accelerated volumetric growth during the early larval development, followed by a reduction in growth in adulthood (CA, RA), which may increase the chance of survival within their native habitat. Indeed, alterations in the dynamics of growth may cause a reorganization and redistribution of energy, such that untreated worms allocate a large proportion of energy to development, while treated nematodes may spend more energy for maintenance and repair functions, thus delaying the aging process.

Third, the intestinal fat deposition was tested (via the fluorescence dye Nile red) and a significant reduction in the fluorescence intensity of Nile red in Q and CA exposed nematodes was found. Nile red (9-diethylamino-5H-benzo[α]phenoxazine-5-one), hailed in the past as an ideal marker for the *in vivo* detection of lipids in *C. elegans*, and has been a valuable tool to elucidate pathways of fat storage and metabolism (Ashrafi et al., 2003). It is well established that Nile Red has negligible effects on growth rate, brood-size, pharyngeal pumping, dauer formation and lifespan (Ashrafi, 2007). Recently, O'Rourke et al. (2009) suggested that the Nile red positive granules within the nematodes intestine are lysosome-related organelles (LROs) and possibly not the major site of neutral fat storage in *C. elegans*. However, since Nile red fluorescence is higher in short-lived mutants, such as *daf-16*, compared to long-lived mutants, e.g. *daf-2*, it was speculated that an increase in staining may be an indicator of decreased stress resistance and a shortened lifespan (O'Rourke et al., 2009). The results obtained in this study strongly support this assumption and suggest that LROs play a role at least in Q and CA induced longevity. Taking all of the fitness and physiological indices into account, these data suggest that the disposable soma theory may at least in part explain the observed longevity effects caused by Q, CA and RA.

## 4.5 Are antioxidative properties *in vivo* responsible for longevity?

The intracellular antioxidative capacity is a key parameter controlling cellular damage and organismic aging. However, experimentally measuring the antioxidative capacity of chemical

compounds *in vivo* is a laborious task. Specifically, antioxidant activity in biological systems cannot be inferred from *in vitro* tests (Trugo et al., 1984; Gazzani et al., 1998a,b). Moreover, antioxidative activity measured *in vivo* in one biological system may not be preserved in another. Although all three PPs have repeatedly proven to possess antioxidant properties *in vitro* (for Review see Rice-Evans et al., 1996), very little exists about their *in vivo* absorption and potential, among them very few or none have been performed with *C. elegans*. Here we demonstrate *in vivo* antioxidant properties of Q, CA and RA in the model organism *C. elegans* and concretize its respective nature by distinguishing between four aspects: (i) impact on lifespan of internal oxidative stressed, hypersensitive *mev-1* mutants; (ii) prevention against paraquat induced matricidal; (iii) TAC in worm homogenate after exposure with respective PP and (iv) fluorescence measurements of lipofuscin levels, as marker for the accumulation of oxidative damaged macromolecules and thus for aging.

This work demonstrates that all three PPs possess *in vitro* antioxidative properties in both hydrophilic and lipophilic solvents (CA > RA > Q). The *in vivo* TAC of water-soluble substances derived from worm homogenates was significantly increased in Q and CA treated samples. The TAC of the worm homogenates reflects the sum of all (water- and/or lipid-soluble substances within the worm that are able to reduce oxygen radicals, which includes PPs metabolites as well as other antioxidants (e.g. glutathione). The comparably low TAC values in RA treated worm samples could be the result of reduced bioavailability and is also supported by the observation that lipofuscin levels are not significantly altered. Konishi et al. (2005) compared the bioavailability of CA and RA in rats. The absorption efficiency was approximately 9.7-fold higher for CA than RA, in accordance with data from human studies (Baba et al., 2005; for detailed review Lafay and Gil-Izquierdo, 2008).

The TAC of lipid-soluble substances in Q, CA and RA treated nematodes, though not significant, resemble trends not unlike those obtained from paraquat induced oxidative stress exposures, suggesting that both phenomena may be interlinked. Since *in vitro* studies have proposed that paraquat exerts its toxicity via redox cycling with the subsequent production of free radicals and oxidative damage to membrane lipids (Smith and Heath, 1976), lipid peroxidation is deemed to be the result of oxidative stress in paraquat exposed organisms (Yasaka et al., 1986; Ogata and Manabe, 1990). Therefore, it is conceivable that Q has the potential to prevent worms from paraquat-induced matricide via lipid-peroxidation, a notion that is in agreement with the free radical theory of aging (FRTA, first proposed by Harman, 1956). Indeed, internal oxidative stress may or may not (the controversy surrounding the FRTA is reviewed by Rattan (2006) and Muller et al., (2007)) resemble a self-amplifying vicious cycle, where the premature disruption of the oxidation chain decreases the risk and magnitude of injury. ROS generation following paraquat treatment takes place in the cytoplasm (Yanase et al., 2002). ROS production in *mev-1* mutants, however, is predominantly produced by a defective electron transport chain located in the mitochondria. This might explain the observed potential of PPs in combating oxidative damage, where differences in chemical nature defines their ability to cross cell membrane barriers and/or their rate of clearance from cells. Indeed, the precise antioxidant properties of PPs *in vivo* will depend on their metabolic pathways within the gastrointestinal tract as well as the biochemical properties of the resulting metabolites. PPs have even been shown to process prooxidant properties (Makris and Rossiter, 2001) which emphasizes that the milieu, the relative amount and bioavailability, are important. Clearly further studies are warranted before the observed effects can be fully interpreted and explained.

Conceivably is furthermore that the observed thermal resistance of at least Q and CA treated nematodes can be explained partly by *in vivo* antioxidative properties: In Q treated animals,

thermal resistance may be also due to hormetic induction of some *hsp*-genes as well as other stress-responsive genes (as shown in the DNA-microarray data). But moreover, will the *in vivo* antioxidative properties (shown by lipofuscin decrease and TAC increase) provoke scavenging of heat-induced ROS, and thereby contribute to enhanced thermal resistance. CA also acts as an antioxidant *in vivo*, but only weakly induces HSP responses. It is conceivable that other CA target genes such as *sir-2.1* or *daf-16* could provoke thermal resistance by activating stress responses. In RA antioxidative properties *in vivo* apparently do not play a major role in stress resistance. However, *hsp* levels are strongly induced in all six tested genes, indicating hormetic overcompensation. Additionally, transcriptional stress responses may be mediated by *sir-2.1* in RA exposed worms.

Taking all findings concerning antioxidative properties together, it appears that

(i) Q is an *in vivo* antioxidant. Following Q exposure the TAC of water-soluble substances in worm homogenate is significantly higher compared to controls. This finding likely correlates with the attenuated lipofuscin levels, as it is thought that *in vivo* antioxidants diminish the rate of oxidative damage, which in turn reduces accumulation of damaged products. The TAC of lipid-soluble substances was also increased and may contribute to preventing paraquat induced matricide. The lifespan of *mev-1* mutants was extended.

(ii) CA is also a potent *in vivo* antioxidant. The highest TAC of water-soluble substances was observed following the exposure to CA, a result that is in agreement with lipofuscin fluorescence being the lowest of all tested conditions. The TAC of lipid-soluble substances resembles control values, a finding that may be in line with results from paraquat challenge.

(iii) RA, although *in vitro* a more potent antioxidant than Q, insignificantly enhanced the TAC of water-soluble substances, and likewise lipofuscin fluorescence was only marginally attenuated. The TAC of lipid-soluble substances rather diminished compared to controls, a finding that may be interlinked to the increase in internal hatch, facing paraquat. It could therefore be argued that RA may act as a prooxidant *in vivo*. Further work needs to be conducted before the observed prolonged *mev-1* lifespan can be fully interpreted.

The present study demonstrates that the *in vitro* antioxidant properties of single compounds do not, by default, match up with an *in vivo* antioxidant impact, a statement that is in sync with Pun et al. (2010). Furthermore, the detection of *in vivo* antioxidant output depends, at large, on the chosen test design (compare for example the results for *mev-1*, TAC and paraquat challenge in RA treated nematodes) and the complexity of the *in vivo* system. Finally, the inclusion of information related to bioavailability and metabolism of compounds would allow a more informed interpretation of results.

## 4.6 Which genetic players promote longevity and stress-resistance?

To investigate the molecular pathways of PP action, we analyzed the longevity promoting effects of PPs in mutant strains. Table 25 provides an overview of the mutants and summarizes known function(s) of mutated genes. Since this work focuses on Q, more mutant strains are tested in this condition. In total, 13 mutant strains were used for Q, 8 for CA and RA, each selected for their involvement in well described stress and/or longevity pathways.

### 4.6.1 Genetic mechanisms of Q's action

**Interpretation of results from mutant lifespan assays**

Four *C. elegans* mutants were identified where Q treatment did not prolong lifespan, nor increase thermotolerance, namely *age-1*, *daf-2*, *unc-43*, and *sek-1*. Some of these results may be unexpected: For example, how is it possible that DAF-2 and AGE-1, both key players in ILS and inhibitors of DAF-16 seem to be responsible for Q-dependent lifespan extension, while *daf-16* isn't? Similarly, why is *daf-16* not involved, even though Kampkötter et al. (2007b, 2008) showed a significant nuclear translocation of the transcription factor DAF-16 following incubation with Q? *daf-16* encodes the central forkhead FOXO transcription factor of the ILS pathway. It is the major downstream effector of DAF-2, the sole *C. elegans* insulin receptor (Tatar et al., 2003). In general, the doubling of life span in *daf-2* mutants is completely dependent on the gene *daf-16* (Kenyon et al., 1993). Additionally to the impact on aging, DAF-16 triggering is crucial for development, stress resistance, thermotolerance, pathogen resistance, metabolism and autophagy. The transcriptional targets of *daf-16* include stress response, antimicrobial, and metabolic genes, as well as many genes of unknown function (for detailed review see Landis and Murphy, 2010), some of which in turn impact aging (Murphy et al., 2003). The high specificity of DAF-16 functional output is in parts regulated by co-factors, which in turn determines which of the variety of DAF-16 targets will be induced (for detailed review see Berdichevsky and Guarente, 2006). In this context, it has been also shown that nuclear translocation of DAF-16, though essential, remains insufficient for transcriptional induction of target genes if cofactors of DAF-16 are missing (Henderson et al., 2006). Thus, lifespan extension due to Q treatment and DAF-16 translocation (shown by Kampkötter et al., 2007b, 2008) coincide, but may not necessarily be causally connected.

In this scenario, alternative mechanisms would underlie the lifespan-prolonging activities of Q, e.g. the antioxidative properties (cf. discussion of *mev-1* mutants). Short-lived *daf-16* mutants are hypersensitive to both thermal and oxidative stress, because they lack the central ILS transcription factor and must carry the burden of elevated internal oxidative stress and partly downregulated innate immune response (Chàvez et al., 2007). The elevated antioxidative activity of Q may attenuate the oxidative stress and thereby causes a physiological gain, resulting in a partial ''rescue'' to native lifespan levels. On the other hand, *daf-2* as well as *age-1* long-lived mutants are armed with robust stress-resistance machineries. It is conceivable that the positive effect of Q could be masked, since oxidative stress resistance and life extension may already be close to their maxima. But how does Q mediated longevity in *akt-2* mutants fit into this picture? Because AKT-2 is located downstream of DAF-2 and AGE-1 in ILS, one may expect *akt-2* mutants not to respond to Q. Recent studies by Hertweck et al. (2004) and Tullet et al. (2008) offer a possible explanation: SGK-1 is a critical control factor in stress response and lifespan, which is activated by ILS independently of AKT/PKB kinase complex. While SGK-1 is the mediator for stress response, AKT/PKB is more important for dauer formation. Hertweck et al. (2004) show that *akt-2* mutants are less stress-resistant than *sgk-1* mutants and in consequence the antioxidative property of Q may still expand the lifespan.

The story is made even more complex by *unc-43* and *sek-1* as they seem to be involved with the molecular mechanism of the Q response. Although both genes were shown to be essential in blueberry polyphenol-derived longevity of *C. elegans* (Wilson et al., 2006), their function cannot be explained by the antioxidative properties of the PPs alone. SEK-1, a MAP2K, is part of the MAP kinase pathway and acts downstream of TIR-1 (toll and interleucin receptor) and NSY-1 (MAP3K). It phosphorylates the *C. elegans* p38-family MAP kinase PMK-1, which results in

elevated immune response to pathogen infection (Kim et al., 2002). NSY-1/SEK-1/PMK-1 cassette also functions via SKN-1 to control resistance against arsenic (An and Blackwell, 2003). SEK-1 is also required for the resistance to oxidative stress by assisting the translocation of cytoplasmic DAF-16 into the nucleus (Kondo et al., 2005). UNC-43 displays a CaMKII ($Ca^{2+}$/calmodulin-dependent protein kinase II). Mutations of the *unc-43* gene result in diverse behavioral abnormalities by affecting locomotory activity, altering excitation of three muscle types, and changing the period of the motor output of a behavioral clock (Reiner et al., 1999). UNC-43, in association with voltage-activated calcium channels (UNC-2/UNC-36), presents a neuronal regulating signaling pathway upstream of TIR-1, NSY-1 and SEK-1, which is described to determine asymmetric *str-2* expression in the AWC olfactory neurons (Sagasti et al., 2001) and thereby influences sensory perception. Sagasti et al. (2001) speculate that the CaMKII-pathway could act in a variety of calcium-dependent processes, like regulation of other cell fates, physiological detection of stress, cell migration, axon guidance and neuronal plasticity. Thus, Q action in *unc-43* and *sek-1* mutants is most likely mediated through altered molecular signaling via the p38 MAP kinase pathway and by sensory perception through olfactory neurons and an up-grade of innate immunity. Further interpretation of these results will be provided in the microarray section.

**Global transcriptional output following Q treatment**

Q possesses a profound impact on transcriptional output and thereby alters the physiological status of *C. elegans*. By performing DNA-microarray experiments and comparing the resulting data with previously published gene expression profiles, an interlaced interplay of genetic pathways affected by Q was revealed. These findings rationalize results obtained in mutant lifespan assays.

*Concentration dependent transcriptional responses:* The DEG numbers in Q 50, Q 100 and Q 200 µM suggest that action of Q in *C. elegans* follows a non-linear mode: A lower concentration (Q 50 µM) regulates processes which potentially improve the health of the nematodes (e.g. the gene classes *gst*s, peroxidases, lysozymes; GO term: oxidoreductase activity; KEGG pathway: glutathione metabolism and metabolism of xenobiotics by cytochrome P450). In line, Q 50 µM induces enhanced thermal stress resistance (data not shown), though it is insufficient to extend the lifespan. For higher Q concentrations between 100 to 200 µM Q, a longevity phenotype is observed and the global transcriptional response of the worms changes substantially. The over-representation of DEGs related to biotransformation (gene-classes: *gst*s, *ugt*s, *cyp*s; KEGG pathways: drug- and xenobiotic metabolism) in $Q_{longevity}$ indicates, that enhanced catabolism of toxic intermediates contributes to the lifespan extension. Thus, Q action resembles predictions made by the green theory of aging, as supported by an additional enrichment of *hsp* expression levels in Q 200 µM. However, further mechanisms likely contribute to Q induced longevity, as indicated by overrepresentation of pdz genes, transcription factors and vitellogenins. The GO term (regulation of transcription, dauer entry, nucleosome and others) and KEGG pathway (Wnt, TGF-beta) results point towards extensive alterations in signaling pathways and transcription. Moreover, provide the alterations of the aminoacid metabolism and certain degradation processes in the lysosome possibilities to contribute to the longevity phenotype.

*Which conclusions can be drawn from this meta-analysis?* The extensive literature comparison allows many conclusions. To address all possibilities is beyond the scope of this work and clearly warrants further investigations. Here only a few aspects can be enlightend.

$Q_{longevity}$ shares significant inducing and repressing transcriptional patterns with long-lived *daf-12(rh273)*, *daf-2* and resveratrol treated *daf-16* mutants as well as nematodes inducing immune response due to infection with *P. aeruginosa* (in parts PMK-1 induced genes). Moreover, $Q_{longevity}$ shares further DEGs with these conditions, even though these genes were found contrarily regulated (up/down or down/up). With TGF-beta mutants, the significant overlap is restricted to induced and also contrary (up in $Q_{longevity}$/down in TGF-beta mutants) regulated genes, as is the case for *pmk-1* mutants (down in $Q_{longevity}$/up in *pmk-1*). Additionally, $Q_{longevity}$ shares overrepresented gene expression mountains with all aforementioned conditions and additionally with dauers. (Noteworthy, since the dauer stage can be induced by ILS and TGF-beta signaling with DAF-12 being the final common target of dauer-regulatory pathways (Hu, 2007), it is not surprising that the transcriptional pattern provoked by $Q_{longevity}$ also shares a significant overlap with dauers.) However, considering all of these results suggests that Q induces transcriptional responses through affecting a complex network of several parallel pathways (schematically depicted in Fig. 30).

The results concerning the involvement of DAF-16 are somewhat contradictive and difficult to interpret: (i) On one hand the comparison with datasets suggests a repression of DAF-16, on the other hand stands the induction of DAF-16 by $Q_{longevity}$. (ii) Furthermore lifespan assays with Q treated *daf-16* mutants revealed, that the lack of DAF-16 does not prevent life extension, whereas the lack of known upstream regulators of DAF-16 (*daf-2* and *age-1*) does. Below follow some enlightening considerations:

(i) A possible explanation for the finding of contrary regulation of DAF-16 (induced vs. repressed) due to $Q_{longevity}$ treatment, is a tissue specific modulation of genetic mediators. For example, it is conceivable that ILS is diminished and thereby DAF-16 induction enhanced in the intestine, but repressed in the somatic gonad. Evans et al. (2008) described already that intestinal and non-intestinal DAF-16 has antagonistic effects on host defense. Furthermore, balanced antagonistic effects on DAF-16 are observed in the context of signaling from the *C. elegans* germline and somatic gonad that regulate aging. While signals from the germline normally suppress DAF-16 activity through ILS, the gonad signaling enhances DAF-16 activity in a parallel pathway that does not require DAF-2 (Arantes-Oliveira et al., 2002). The output of this balanced signaling can be described as net aggregate effect (Evans et al., 2008). The complex regulation of DAF-16 with co-regulators which influence lifespan and stress resistance together with DAF-16 (Hsu et al., 2003; Wolff et al., 2006; Tullet et al., 2008), could possibly further explain this phenomenon. The exact mechanisms to which degree and in which tissue $Q_{longevity}$ activates and represses DAF-16 activity should be addressed in future research.

(ii) Q 200 µM was not able to extend the lifespan of *daf-2* and *age-1* mutants (Pietsch et al., 2009). The corresponding proteins are key players of the ILS pathway and, obviously, also essential for lifespan extension due to Q. Longevity in *daf-2* mutants is thought to be largely dependent on the lack of inhibiting DAF-16 (reviewed in Kurz and Tan, 2004). Surprisingly however, treatment with Q 200 µM provoked a robust life extension of 15% in *daf-16* mutants, which even exceeds results for the wild type (+ 10%). Thus, despite of the big proportion of DAF-16 dependent transcriptional responses due to $Q_{longevity}$ in wild type worms (shown by regulated genes e.g. *sod-3*, *sodh-1*, other biotransformation genes and the significant overlap to *daf-2* mutants in general), it is suggested that DAF-16 mediated transcritption is not responsible for life extension. This phenomenon presumably becomes explainable by considering the p38 MAPK-pathway as a further participating component, as proposed by Pietsch et al. (2009) due to missing lifespan extension following Q 200 µM exposure in *sek-1* and *unc-43* mutants, respectively. This underlines the importance of p38 MAPK pathway, which is presumably mod-

ulated by TGF-beta and ILS (Kurz and Tan, 2004) or others like DAF-12 or also germline/gonad signaling. The *sek-1* gene encodes a MAPKK, part of the MAP kinase pathway, acting downstream of TIR-1 and NSY-1, which phosphorylates the p38-family MAP kinase PMK-1, resulting in elevated immune response to pathogen infection (Kim et al., 2002). Troemel et al. (2006) proposed this PMK-1 dependent pathway to act downstream of *daf-2* and in parallel to ILS. Indeed, DEGs up-regulated in response to *P. aeruginosa* infection correlate highly with genes determined as induced by PMK-1 (Troemel et al., 2006). Moreover, also $Q_{longevity}$ DEGs show substantial overlap to genes being PMK-1 targets. All these results suggest that Q treatment regulates DAF-2 activity on the protein level, which in turn regulates DAF-16 and PMK-1 (possibly already upstream of PMK-1, e.g. *unc-43* and *sek-1*). DAF-16 modulates thereby rather the general stress response, whereas PMK-1's activity leads to a stronger immune response. Both pathways concurrently provoke an interplay of induction and repression of their target genes, which partly overlap (e.g. CUB-like genes and C-type lectins, which are down-regulated in *daf-2* mutants but up-regulated following infection with *P. aeruginosa*). The fact that some CUB-like genes are also down-regulated in *daf-12(rh273)* points toward a regulatory role for DAF-12, too. Why CUB-like genes are down-regulated by DAF-16, even though they promote immunity, is unclear. Troemel et al. (2006) suggested that FOXO transcription factors have opposing roles in the immune response; while promoting certain kinds of immunity, inhibiting others. Possibly, this subtle down-regulation of certain immune responses contributes for the life prolonging effects of DAF-16 in *daf-2* mutants grown on *E. coli*. Immune response proteins might be energetically costly and unnecessary when grown on a relatively non-pathogenic food source. This can be also the reason for the clear gain in mean lifespan due to Q 200 µM exposure in *daf-16* mutants compared to wild type nematodes. The usually induced general stress response is partially turned off, while simultaneously the immune response through p38 MAPK (and probably other mechanisms) becomes activated. Conceivable, is the abolishment of this part extinction in the case of *daf-16* mutants, which saves energy and prolongs lifespan over the limit of wild type nematodes (Pietsch et al., 2009). In this scenario, the suppression of CUB-like genes and other immune effectors by DAF-16 on one hand and the upregulation of other immune genes by PMK-1 counterbalance the optimal energy consumtion and protection against pathogens in this scenario. The regulation of gene expression by Q seems to be an array of several ancillary activities, as an inactivation of ILS and an increase of stress resistance, an enhanced immune response and an involvement of DAF-12. In this way, Q action in *C. elegans* is an interesting example of how pathways interact, both in a cumulative and also in an antagonistic way; mechanisms which deserve closer attention in future investigations.

However, as mentioned before, it is also conceivable that the lifespan results of *daf-16* mutants are somewhat deceiving: Possibly the internal status of these short-living worms, lacking this important transcription factor, have a high ground level of stress and ROS and thus are highly susceptible for the antioxidative properties of Q. In this scenario, the antioxidative properties *in vivo* would overwhelm and mask the genetic effects of Q. In this case the commonly used interpretation of lifespan results would be delusive. Furthermore, it should be noted that DNA-microarray assays can also be erroneous and should be taken with caution. Mistakes in breeding and harvesting of the worms and in RNA preparation, DNA-microarray hybridization and evaluation of the data can cause false results. Hence, the comparison of datasets from various studies therefore can be misleading. Confirming of found results by other tests like RNAi of in DNA-microarrays identified important genes, further mutant assays (lifespan and DNA-microarrays) or overexpression experiments will have to be conducted in the future.

Nevertheless, the regulation of gene expression by Q seems to be an array of several ancillary activities, as an inactivation and/or activation of ILS and an increase of stress resistance, an

enhanced immune response and an involvement of TGF-beta signaling, DAF-12, and furthermore presumably germline signaling. In this way, Q action in *C. elegans* is an interesting example of how pathways get influenced by nutrition and how they obviously interact, both in a cumulative and also in an antagonistic way.

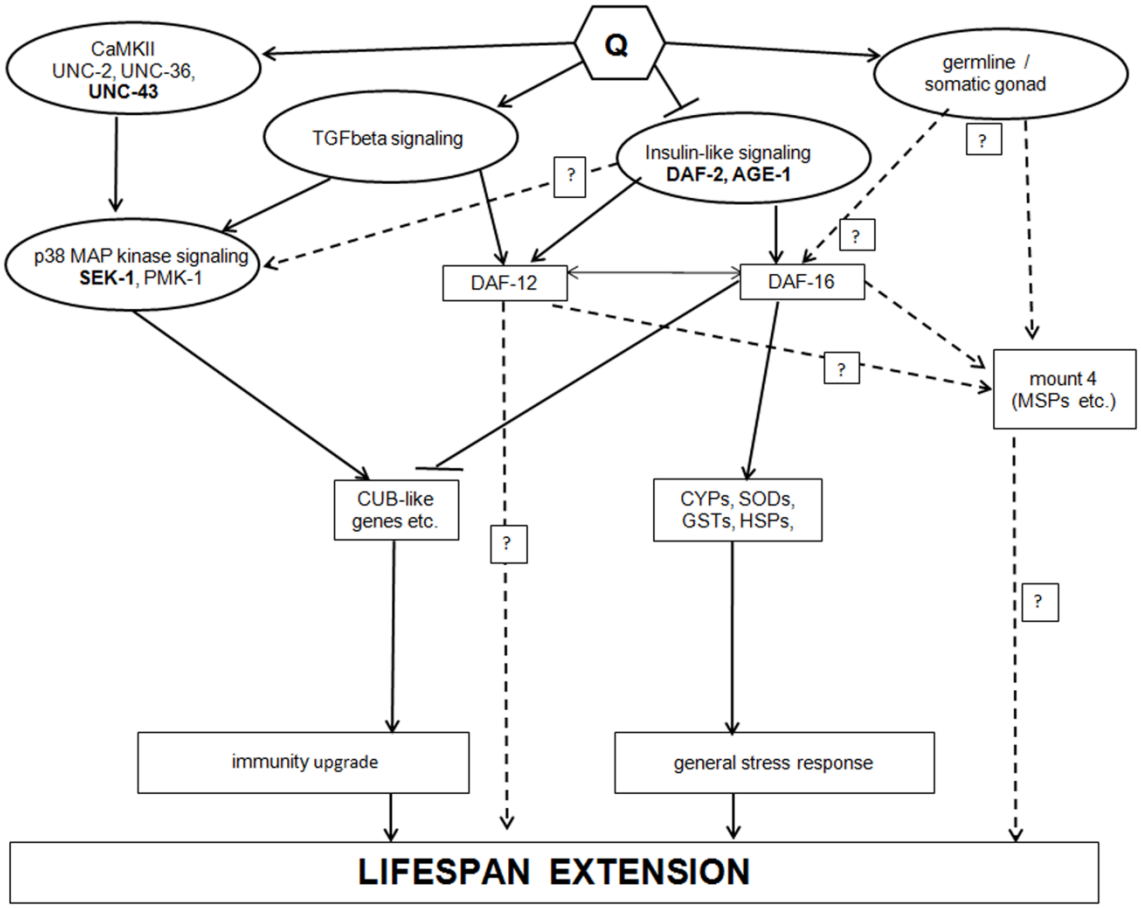



**Fig. 30 Presumable model of Q's lifespan extending genetic action *in vivo.*** Dottet lines point out unknown types of interactions.

### 4.6.2 Genetic mediators of CA's and RA's action: mutant lifespan assays

The models in Fig. 31 A and B give a schematic overview about CA's and RA's action. As previously identified for Q, *sek-1* and *unc-43* are key players in CA and RA mediated longevity. In addition, *osr-1* was shown to be required, a finding that resembles results obtained by Wilson et al. (2006) on blueberry polyphenol induced longevity. As far as known, OSR-1 regulates osmotic stress response and survival in hyper-osmotic environments. Viability under osmotic stress depends on the activity of several proteins of the CaMKII pathway, where OSR-1 is coupled to SEK-1 through UNC-43 (Solomon et al., 2004). The re-occurring finding that *osr-1*, *unc-43* and *sek-1* are involved in PPs mediated lifespan extension provides compelling evidence that a, yet undefined, pathway exists, although there are notable exceptions: Neither *osr-1*, *unc-43* nor *sek-1* were shown to be responsible for lifespan enhancement in catechin exposed worms, nor *osr-1* and *unc-43* in tannic acid exposed nematodes (Saul et al., 2009; 2010). However, the CA and RA induced interplay of these three genes, presumably leads to

**Table 25 Overview of genes tested in mutant lifespan assays for involvement in Q's, CA's and/or RA's life extending action modes.** Grey fields indicate genes that have been only investigated for Q's involvement. Second column displays found involvement in respective PP action modes.

| | | |
|---|---|---|
| ***age-1*** | Q | - ortholog of the phosphoinositide 3-kinase (PI3K) p110 catalytic subunit<br>- central component of the *C. elegans* ILS pathway, downstream of DAF-2/insulin receptor and upstream of both the PDK-1 and AKT-1/AKT-2 kinases and the DAF-16 forkhead type transcription factor<br>- required for regulation of metabolism, life span, dauer formation, stress resistance, salt chemotaxis learning, fertility, and embryonic development<br>- Mutations result in long-lived nematodes, due to missing inhibition of DAF-16 translocation |
| ***akt-2*** | | - Negative modulator of DAF-16 in ILS (Hertweck et al., 2004, Baumeister et al., 2006) |
| ***daf-12* (daf-d)** | | - Promotes gonad-dependent adult longevity (Gerisch et al., 2007); tested was the daf-d m20 allell, which provokes a shorter lifespan than wild type |
| ***daf-16*** | CA | - central forkhead transcription factor of ILS, major downstream effector of DAF-2 (Tatar et al., 2003).<br>- doubling of lifespan in *daf-2* mutants is completely dependent on *daf-16* (Kenyon et al., 1993)<br>- crucial for development, stress resistance, thermotolerance, pathogen resistance, metabolism, and autophagy<br>- transcriptional targets: stress response, antimicrobial, and metabolic genes, many genes of unknown function (for detailed review see Landis and Murphy, 2010), some of them impact aging (Murphy et al., 2003)<br>- regulated by co-factors, which determine its functional specificity (for detailed review see Berdichevsky and Guarente, 2006). |
| ***daf-2*** | Q | - central player in ILS<br>- predicted receptor tyrosine kinase that is the *C. elegans* insulin/IGF receptor ortholog;<br>- required for a number of processes in *C. elegans*, including embryonic and larval development, formation the dauer larval stage, adult longevity, reproduction, fat storage, salt chemotaxis learning, and response to exogenous stressors such as high temperature or bacterial infection;<br>- signals through a conserved PI 3-kinase pathway to negatively regulate the activity of DAF-16, by inducing its phosphorylation and nuclear exclusion<br>- mutations result in long-lived nematodes, armed with a robust stress-resistance machinery due to missing inhibition of DAF-16 translocation, resulting in increased expression of its manifold target genes<br>- negatively regulates the nuclear localization, and hence transcriptional activity of SKN-1 in intestinal nuclei |
| ***jnk-1*** | | - Post-translational positive regulation of DAF-16 (Wolf et al., 2008) |
| ***mev-1*** | | - Mutants showed significant PP-mediated gain in lifespan. This mutant is characterized by an elevated accumulation of endogenous ROS (Ishii et al., 1998) and provides a special test system to prove an antioxidative capacity. |
| ***nhr-8*** | | - active in the xenobiotic defense system (Lindblom et al., 2001) |
| ***sek-1*** | Q<br>CA<br>RA | - MAP2K, part of the MAP kinase pathway, downstream of TIR-1 (toll and interleucin receptor) and NSY-1 (MAP3K)<br>- phosphorylates the *C. elegans* p38-family MAP kinase PMK-1, which results in elevated immune response to pathogen infection (Kim et al., 2002).<br>- NSY-1/SEK-1/PMK-1 cassette also functions via SKN-1 to control resistance against arsenic (An and Blackwell, 2003) |
| ***unc-43*** | Q<br>CA<br>RA | - CaMKII ($Ca^{2+}$/calmodulin-dependent protein kinase II), mutations causes diverse behavioral defects by affecting locomotory activity, altering excitation of three muscle types, and changing the period of the motor output of a behavioral clock (Reiner et al., 1999).<br>- in association with voltage-activated calcium channels (UNC-2/UNC-36), presents a neuronal regulating signaling pathway upstream of TIR-1, NSY-1 and SEK-1, determines asymmetric str-2 expression in the AWC olfactory neurons (Sagasti et al., 2001) |
| ***osr-1*** | CA<br>RA | - thought to be involved in the regulation of osmotic stress response and the survival in hyper-osmotic environments<br>- is coupled to SEK-1 through UNC-43 (Solomon et al., 2004). |
| ***sir-2.1*** | CA<br>RA | - $NAD^+$-dependent deacetylases (North and Verdin 2004)<br>- implicated in the regulation of aging and longevity across a wide variety of organisms(Tissenbaum and Guarente, 2001).<br>- responsive to metabolic changes in the cellular environment, including nutrient/energy availability and cellular stress (Lin and Guarente, 2003).<br><u>suggested working modes:</u><br>- *sir-2.1* requires *daf-16* to extend the lifespan of *C. elegans* via ILS (Tissenbaum and Guarente, 2001)<br>- SIR-2.1 and 14-3-3 act parallel to ILS in a stress-dependent pathway, activate DAF-16 and thus extends lifespan (Berdichevsky et al., 2006).<br>- *sir-2.1* may (Tissenbaum and Guarente, 2001; Wood et al., 2004) or may not (Houthoofd and Vanfleteren, 2006; Kaeberlein et al., 2006) promote longevity due to caloric restriction (CR); (there is sufficient evidence from yeast, fly and worm experiments that SIR2 is involved in lifespan extension by CR that is independent of the ILS pathway (Lin et al., 2000; Rogina and Helfand, 2004).<br>- *sir-2.1* and *daf-16* have overlapping but also distinct roles in lifespan regulation (Wang and Tissenbaum, 2006).<br>- Viswanathan et al. (2005) demonstrated that resveratrol mediates the lifespan extension of *C. elegans* through a *sir-2.1*-dependent ER stress pathway.,via the up-regulation of a family of stress response genes (especially *abu-11*), which are thought to aid in protein folding within the endoplasmic reticulum (Urano et al., 2002) and thus is independent of *daf-16*. Resveratrol, in turn, inhibits SIR-2.1, which suppresses the expression of *abu/pqn* genes, thereby leading to de-repression of *abu-11* and other *abu/pqn* genes. The result is an elevated level of protein folding surveillance within the ER and results in an increase of lifespan in the nematode. |
| ***skn-1*** | | - Developmental transcription factor, responsible for the expression of various stress genes promoting oxidative stress resistance, shows striking similarities to DAF-16 mediated stress response, so far no regulatory link to IIS has been identified (An and Blackwell, 2003; Inoue et al., 2005; Baumeister et al., 2006) |

longevity through different outputs: (i) an elevated immune response to pathogens, (ii) stress-detection and stress-response and (iii) regulation of cell fates.

In addition to the *osr-1*/*unc-43*/*sek-1* pathway, *daf-16* and *sir-2.1* were also found to be involved in CA induced longevity, since no lifespan extension was observed in CA exposed *daf-16* and *sir-2.1* mutants. The Sir2 family of $NAD^+$-dependent deacetylases (Imai et al., 2000; Landry et al., 2000; North and Verdin, 2004) has been implicated in the regulation of aging and longevity across a wide variety of organisms, including yeast, worms and flies (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004). SIR2 and its orthologs are responsive to metabolic changes in the cellular environment, including nutrient/energy availability and cellular stress (Lin and Guarente, 2003). In *C. elegans*, genetic analysis initially suggested that *sir-2.1* extends life span via the ILS pathway and depends on *daf-16* (Tissenbaum and Guarente, 2001). Later, Berdichevsky et al. (2006) proposed the existence of a stress-dependent pathway in which SIR-2.1 and 14-3-3 act in parallel to the ILS pathway, but still emerge on and activate DAF-16, to extend life span. 14-3-3 proteins are highly conserved small acidic proteins, which bind phosphoserine and phosphothreonine residues in a context-specific manner (Durocher et al., 2000) and rank among DAF-16 cofactors. Furthermore, although controversially discussed, *sir-2.1* may (Tissenbaum and Guarente, 2001; Wood et al., 2004) or may not (Houthoofd and Vanfleteren, 2006; Kaeberlein et al., 2006; Lee and Lee, 2006) promote longevity during caloric restriction (CR). Regardless, studies with certain yeast strains, flies and worms points toward an involvement of SIR2 in life span extension by CR, independent of the IIS pathway (Lin et al., 2000; Rogina and Helfand, 2004; Wang and Tissenbaum, 2006). Hence, it has been proposed that *sir-2.1* and *daf-16* play overlapping, as well as distinct roles in life span regulation (Wang and Tissenbaum, 2006). Moreover, acetylation and deacetylation of chromatin-interacting proteins is central to the epigenetic regulation of genome architecture and gene expression. Due to the emerging realization of the pathogenetic role of histone deacetylation in human disorders, a great deal of effort has been evolved to detect chemicals that modulate histone deacetylases activity (Johnstone, 2002; Grozinger and Schreiber, 2002). Howitz et al. (2003) showed that sirtuin activating compounds (STACs) like resveratrol – a polyphenol- and various other small molecules can promote the survival of human cells and extend the replicative lifespan of yeast. Viswanathan et al. (2005) demonstrated in genomic and genetic analyses conducted with *C. elegans*, that resveratrol mediate lifespan extension through a *sir-2.1*-dependent ER stress pathway. The mechanism involves up-regulation of a family of stress response genes (especially *abu-11*), which are thought to aid protein folding in the endoplasmic reticulum (Urano et al., 2002) and thus is independent of DAF-16. Resveratrol evidently acts to inhibit SIR-2.1, which under normal conditions suppress the expression of *abu/pqn* genes, thereby leading to de-repression of *abu-11* and other *abu/pqn* genes. The result is an elevated level of protein folding surveillance within the ER that, in turn increases the life span of the nematode (Viswanathan et al., 2005).
Given that the lifespan of *daf-2* and *age-1* mutants is positively affected by CA treatment, it is likely that CA operates through a SIR-2.1, 14-3-3 and DAF-16 pathway that is independent of ILS. CA may, in addition, promote longevity by means of a *sir-2.1*-dependent ER-stress response pathway and/or the presumable CR-pathway. In contrast, RA triggered life extension is seemingly unconnected to *daf-16* and presumably controlled by a *sir-2.1*-dependent ER-stress-pathway, the putative *sir-2.1*-dependent CR-pathway, or through a yet to be discovered molecular switch.

Three questions remain: (i) Why is CA's but not RA's life prolonging action dependent on *daf-16*? Results obtained from rats suggest that RA is partially hydrolyzed to CA (Baba et al. 2004); it is therefore conceivable that CA may also be produced in *C. elegans* following the ingestion

and hydrolyzation of RA. Clearly, further metabolic and genetic investigations are needed to allow firm conclusions to be made. (ii) Why does CA exposure induce negative lifespan results in *osr-1* and *sir-2.1* mutants? (iii) If the mutants already have high base line levels of internal stress (due to their physiological status), would additional external influences (such as PP exposure) not interfere with their ability to respond to stress? The latter question raises an important, and often overlooked, point: namely that the underlying internal physiology should not be neglected when interpreting lifespan assays with mutant animals.

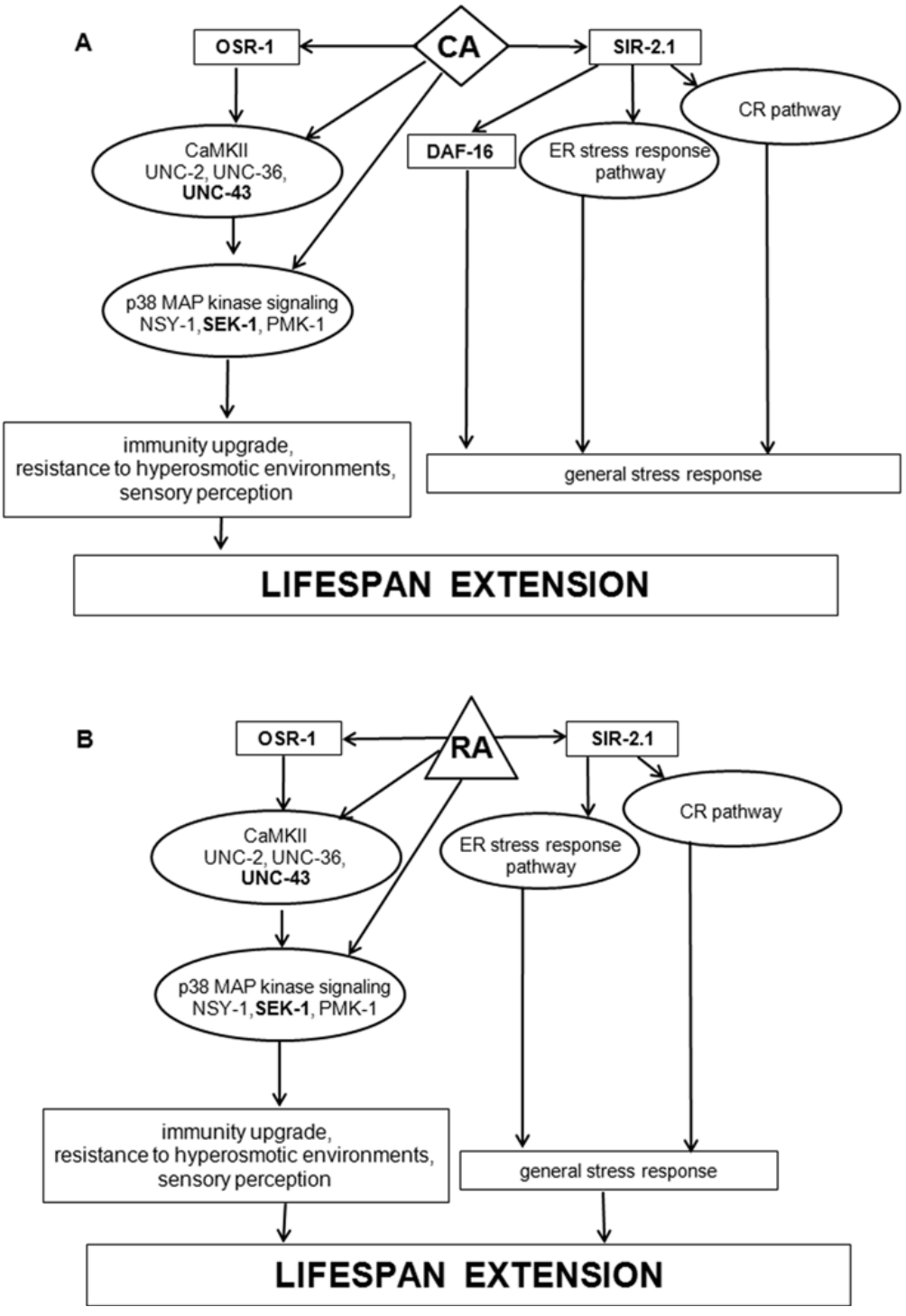



**Fig. 31 Presumable models of (A) CA's and (B) RA's genetic working modes**

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## 4.7 Do Q, CA and RA use common or exclusive mechanisms?

Table 26 summarizes the main results of this thesis and compares the effects of the three PPs. All three PPs share common but also display specific/exclusive mechanisms:

Q, CA and RA all induce hormetic dose-responsive life extension in *C. elegans*. However, because of the differences in the underlying activation of stress responses (as defined by changes in *hsp* expression), only Q and RA are arguably classical hormetins. When taking into account the results from mutant lifespan assays, it is conceivable that CA provokes a *sir-2.1* and/or *daf-16* dependent stress-response (ER-stress response or CR-stress response) in a hormetic sense and thus also could be classified as hormetin.

The antimicrobial properties of Q and RA were not deemed to be the main reason for the life prolonging action, since the lifespan of wild type nematodes was still expanded when grown on heat killed *E. coli* supplemented with either Q or RA. CA did not display an antibacterial property at all.

Furthermore, different *in vivo* antioxidative properties of all three PPs suggested the presence of distinct modes of action, presumably dependent on their metabolism, ability to cross cell membrane barriers and clearance from cells. Further tests are needed to define the exact underlying mechanisms.

Considering experiments in mutant strains revealed also common and distinct genetic mechanisms. All three PPs share the involvement of *sek-1* and *unc-43*, what implicates the participation of the p38 MAPK pathway, as well as the CaMKII pathway. It is apparent that *C. elegans* recognizes all three PPs and activates an immune response pathway previously thought to be limited to pathogen infection. CA and RA additionally employ OSR-1 and SIR-2.1; OSR-1 mediates resistance to hyperosmotic environments and SIR-2.1 was shown to activate stress response in the ER and possibly controls CR. DAF-16 appears to contribute to CA action (apparently independent from ILS), but plays no role for RA. In contrast, Q presumably uses the ILS and the TGF-beta pathway by signaling through DAF-12, but the longevity phenotype is essentially independent of DAF-16.

Neither Q, nor CA or RA provoke direct CR by effective lowering of food ingestion (as shown by the attraction assay and the pharyngeal pumping rates), but indirect CR by involvement of SIR-2.1 cannot be excluded in the cases of CA and RA. A reallocation of energy takes obviously place due to all three PPs, but with varying implications.

Overall, these findings point towards a complex variety of underlying mechanisms – some in common, some differing.

**Table 26 Summary of results from conducted experiments** with Q, CA and RA. If not otherwise stated experiments are performed with Q 200 µM, CA 300 µM and RA 200 µM at 20 °C. Changes were calculated compared to control (0 µM). "–" indicates a decrease, "+" a increase, "/" stands for non-significant results. "(–)" or "(+)" spotlight non-significant tendencies.

| | Experiment | Additional information | Q | CA | RA |
|---|---|---|---|---|---|
| **Artifacts due to test design** | Antimicrobial properties | Lifespan extension on heat-killed OP50 (change) | 1.09±0.01** | 1.15±0.03** | 1.22±0.02** |
| | | Bacterial growth inhibition | -** | / | -* |
| | Transgenerational effects | Adaptive<br>Additive | /<br>/ | N/A | N/A |
| **Hormesis and stress resistance** | Concentration-dependence of lifespan variations (change) | 50 µM<br>100 µM<br>200 µM<br>250 µM<br>300 µM<br>600 µM | 1.02±0.00<br>1.11±0.06**<br>1.10±0.03**<br>0.93±0.03* | 1.00±0.01<br>1.09±0.05**<br>1.07±0.04*<br>1.11±0.02**<br>1.06±0.03** | 1.02±0.01<br>1.06±0.01<br>1.10±0.03**<br>1.08±0.03**<br>0.94±0.01* |
| | Pinpointed genes responsible for PPs action | Mutant lifespan assays | *age-1*, *daf-2*, *sek-1*, *unc-43* | *daf-16*, *osr-1*, *sek-1*, *sir-2.1*, *unc-43* | *osr-1*, *sek-1*, *sir-2.1*, *unc-43* |
| | Presumable pathways | **Q:** deduced from microarray and mutant lifespan results<br>**CA, RA:** deduced from mutant lifespan assays | TGF-beta<br>ILS<br>P38 MAPK<br>CaMKII | p38 MAPK<br>CaMKII<br>ER/CR stress response | p38MAPK<br>CaMKII<br>ER/CR stress response |
| | Thermotolerance | | +** | +** | +* |
| | *hsp* expression | | 4 up<br>2 down | 1 up<br>5 down | 6 up |
| **Antioxidative Capacities *in vivo*** | Paraquat assay: matricidal prevention (change) | | 4.8x** | 1.2x | 0.29x** |
| | Antioxidative capacity in worm homogenate | TAC water-soluble<br>TAC lipid-soluble | +*<br>/ (+) | +*<br>/ | /<br>/ (-) |
| | *mev-1* lifespan extension (change) | | 1.10±0.06** | 1.10±0.02** | 1.08±0.04** |
| | Lipofuscin levels | | -* | -* | / (+) |
| **Reallocation of resources** | Growth | Heat-killed at the 6$^{th}$ day of old adults: length (µm)<br>Alive larvae (0 - 36 h post-hatching): area (µm$^2$) | /<br>+* | -**<br>+** | -**<br>+** |
| | Lipid metabolism in LROs | | -* | -* | / (+) |
| | Reproduction | Total brood size<br>Delay | /<br>/ (-) | /<br>/ (-) | /<br>-* |
| **Direct and indirect CR effects** | Pharyngeal pumping on certain days of adulthood | 3$^{rd}$ adult day<br>6$^{th}$ adult day<br>10$^{th}$ adult day | +**<br>+**<br>+** | +**<br>+**<br>+** | +**<br>+**<br>+** |
| | Attraction | | / (-) | / | / (-) |
| | *sir-2.1* lifespan (change) | | 1.08**±0.03 | 0.93*±0.00 | 1.03±0.01 |
| | Lifespan without OP50 (change) | | 1.10**±0.03 | N/A | N/A |

## 4.8 Is it possible to apply common aging theories to the results of this study?

Different theories approach aging from different viewpoints (e.g. mechanistic vs. evolutionary). Nevertheless, aging theories frequently overlap in certain aspects and in the predictions made (or at least they do not contradict each other). It is thus difficult to experimentally discriminate between aging theories. Nevertheless, the following discussion tries to integrate findings of the present study into the framework of popular aging hypotheses. It will mainly focus on mechanistic approaches (except the disposable soma theory), since evolutionistic theories are difficult to confirm and mainly based on processes which define the maximum lifespan of species, as opposed to individuals. Table 27 summarizes aging modulations and theories which can be utilized to explain the working mode of each PP. Predictions made for hormesis embrace and resemble at large the Green theory of aging. Moreover, it is suggested that mechanisms which underlie CR can be explained by hormetic working modes (cf. Introduction) and thus, also follow statements made by the Green theory. In this context, hormesis and CR are discussed together with the applicability of aging theories.

*Green theory of aging, hormesis, xenohormesis, mitohormesis and indirect CR:* All three PPs operate on hormetic working schemes. More exact as xeno-hormetins, since all derive from nutritional sources and produce stress responses in *C. elegans* and thereby provoke longevity phenotypes. Gene expression profiling supports that Q-induced longevity follows the Green theory of aging, since biotransformation - and *hsp*- genes are overrepresented in Q treated nematodes. Likewise, induction of all inspected *hsp*s by RA suggests that this PP might operate in a Green theory manner as well. Since CA brought no significant induction of examined *hsp*s in this study and no other biotransformation genes have been examined, clear evidence for the application of the Green theory to CA's action could not be found. The lack of lifespan extension in *sir 2.1* mutants suggests that CA and RA may employ a *sir 2.1* dependent signaling pathway to induce: (i) an indirect CR effect, thus acting as CR mimetic or (ii) stress responses, both presumably involving hormetic working modes, and thus could in turn confirm the Green theory of aging.

However, it should be noted, that hormesis in its original sense implies that there are no negative side-effects of the actual positive effect. Since this study examined only a few parameters under standardized laboratory conditions, it cannot be excluded that major negative effects have been overlooked. In this context, it is not possible to estimate whether the observed alterations in physiology of the nematodes (e.g. delayed reproduction, altered size, altered fat content) under laboratory conditions, could have negative influences on overall fitness in natural ecological environments. Therefore it is difficult to conclude that the inverted U-shaped dose-response curves reflect true hormesis.

*Free Radical- and Mitochondrial theory of aging vs. Mitohormesis:* Considering the fact that Qs' and CAs' antioxidative *in vivo* actions provoke a reduced lipofuscin level, and Q additionally diminishes paraquat pressure on reproducing hermaphrodites, one could state that both PPs decrease the internal ROS level and thereby promote longevity. This would confirm predictions made by the FRTA. It seems that the lifespan prolongation of *mev-1* mutants results from diminished production of ROS by the mitochondria or enhanced ROS scavenging in the mitochondria following treatment with all three PPs. Hence, this would be corroboration for the MTA. On the other hand, it could be argued that RA which acts as a prooxidant *in vivo* employs mitohormetic actions; by increasing ROS production in the mitochondria it might provoke an enhanced induction of stress responses and thereby promote life extension. Further studies are required to discriminate these hypotheses.

*Diposable Soma like patterns:* Since redistribution of resources occurs in Q, CA, and RA-treated animals, the Disposable Soma theory could be applied to all three PPs. Specifically, we observed altered developmental time spans, altered adult growth (in CA and RA) and altered fat metabolism (Q and CA). It is conceivable that the PPs induce a shift of energy towards improved maintenance functions (e.g. stress response, biotransformation) with concurrently taking a loss in subordinary parameters (like growth in adulthood); this would finally improve the reproductive success by ensuring a better health status of the individual, and the enhanced longevity would merely be a side-effect of improved health.

**Table 27 Hypotheses about aging which can be applied to findings due to respective PP treatment**

| Q | CA | RA |
|---|---|---|
| **Green theory of aging** | | |
| *(Xeno-) Hormetic working modes*<br>- HSP Induction<br>- Biotransformations genes induced (*cyp*s, *gst*s, *ugt*s, *sod*s) | *(Xeno-)Hormetic working modes*<br>- Indirect CR (by means of *sir-2.1* induced ER or CR stress response) | *(Xeno-)Hormetic working modes*<br>- HSP induction<br>- Mitohormesis by prooxidatve properties (enhanced paraquat susceptibility, slightly enhanced TAC of lipid-soluble substances)<br>- indirect CR (by means of *sir-2.1* induced ER or CR stress response) |
| **Free Radical- and Mitochondrial theory of aging** | | |
| *Antioxidative properties (FRTA; MTA)*<br>- Reduced lipofuscin levels<br>- Enhanced TAC of water soluble substances in worm-homogenate<br>- Reduction of paraquat induced "bagging phenotype<br>- *mev-1* lifespan extension | *Antioxidative properties* (FRTA, MTA)<br>- Reduced lipofuscin levels<br>- Enhanced TAC of water soluble substances in worm-homogenate<br>- *mev-1* lifespan extension | *(Antioxidative properties (MTA)*<br>- *mev-1* lifespan extension ) |
| **Disposable Soma** | | |
| *Reallocation of resources*<br>- Larval growth increased<br>- Slight delay of onset of reproduction<br>- Reduced fat content in LROs | *Reallocation of resources*<br>- Larval growth increased<br>- Adult growth decreased<br>- Slight delay of onset of and shift during reproduction<br>- Reduced fat content in LROs | *Reallocation of resources*<br>- Larval growth increased<br>- Adult growth decreased<br>- delay in onset and shift during reproduction |

# 5 Conclusion

## 5.1 What can be learned from this study?

Studying phytochemical actions in *C. elegans* is the first step to understand the complexity of underlying mechanisms in a multicellular organism. Although further detailed investigations are required, results of this study provide first insights into Q's, CA's and RA's multifaceted functions *in vivo*. Taken together, our findings suggest that the PPs are able to target aging throughout life, rather than just being limited to improving survival at old age. This theory is supported by the increase in pumping rates, which typically decline in older animals, the attenuated lipofuscin accumulation, the diminished Nile red fluorescence and the changes in reproductive timing. Q can be best classified as an *in vivo* antioxidant with strong hormetic potential. CA is also a potent *in vivo* antioxidant, while RA more likely acts as a prooxidant with hormetic working mode.

### 5.1.1 General working scheme of PP action

This comparative study identified common and contrasting mechanisms underlying longevity-promoting actions of Q, CA, and RA. By various bioassays and DNA-microarray analyses the PPs were shown to have a profound impact on the *in vivo* status of *C. elegans*. Various aspects of nematode life are measurably affected: stress resistance, lifespan, growth, development, fat metabolism, food up-take, the internal TAC levels and genetic signaling cascades. It is likely that Q, CA and RA affect additional physiological parameters, implying that further mechanistic studies are required to fully understand PP action. Similar, drastic physiological alterations were previously reported in *C. elegans* treated with other natural compounds and antioxidants (reviewed Gruber et al., 2009; Saul et al., 2009). In conclusion, it seems that many phytochemicals are able to reduce age-related declines and produce changes in physiological parameters, although not all studies found life extension (summarized in Supplementary Table 16, taken from Saul et al., 2009).

Figure 32 depicts a presumable general working scheme for PPs *in vivo*. To a different extent PPs act through genetic signaling, hormetic and antibacterial working modes, anti- or pro-oxidative properties *in vivo* and by regulation of the metabolism. The relative contribution of each effect may depend on the generic structure and bioavailablility of respective PP, as well as the treated organism, its developmental and physiological state, the examined tissue and other external influences. The direct responses to PPs in turn influence each other, giving rise to a complex feedback and feed-forward network (Fig. 32). Future studies might refine the simplified model in Fig. 32 which is only a crude approximation of the real network. The present study did not address the hierarchy of events within this network, an aspect that deserves further investigation

As example, it is conceivable that PPs provoke a slight physiological stress when ingested, like environmental xenobiotics. Adequate genes will be induced through genetic signaling. These reactions will be also categorized as hormetic working modes, if the biotransformation system, stress response or immunity genes are involved. Some biotransformation genes play also roles in lipid metabolism (e.g. some *cyp*-genes) and their activation may provoke altering of the lipid content and thereby change the internal ROS level. When inducing immunity genes, the antimicrobial defense of the organism will increase. Vice versa can direct antimicrobial properties of the PP also upgrade the overall immunity, by straightforward combating invading microbes. Less pathogen stress will reduce in turn the ROS generation. Conceivable is also, that the prooxidant or antioxidant properties of the PP will exert direct or indirect effects on the oxidative

status: internal ROS will be increased or decreased. Enhanced levels of ROS can help also in pathogen defense and furthermore were shown to act as signaling molecules (Lamb and Dixon, 1997), provoking presumably mitohormesis. Beside will parts of the metabolism convert the PP in its metabolites which can induce further genetic signaling, activate again parts of the metabolism, induce again hormetic working modes what presumably can lead again to ROS increase or decrease and so forth. Conceivable are many more interlinks and feedback loops.

Although all phytochemicals will employ one or several of the network branches in Fig. 32, the ultimate output is not necessarily life extension and stress resistance. For instance, during the course of this study other compounds were tested, including cumaric acid, vanillic acid and resveratrol (data not shown); no life extension could be shown for each of these agents over a broad concentration range. Thus, it is likely that the physiological response to phytochemicals is determined by the combination of molecular events. It will stay still a major challenge for future studies to further define the relation between molecular events and organismic aging.

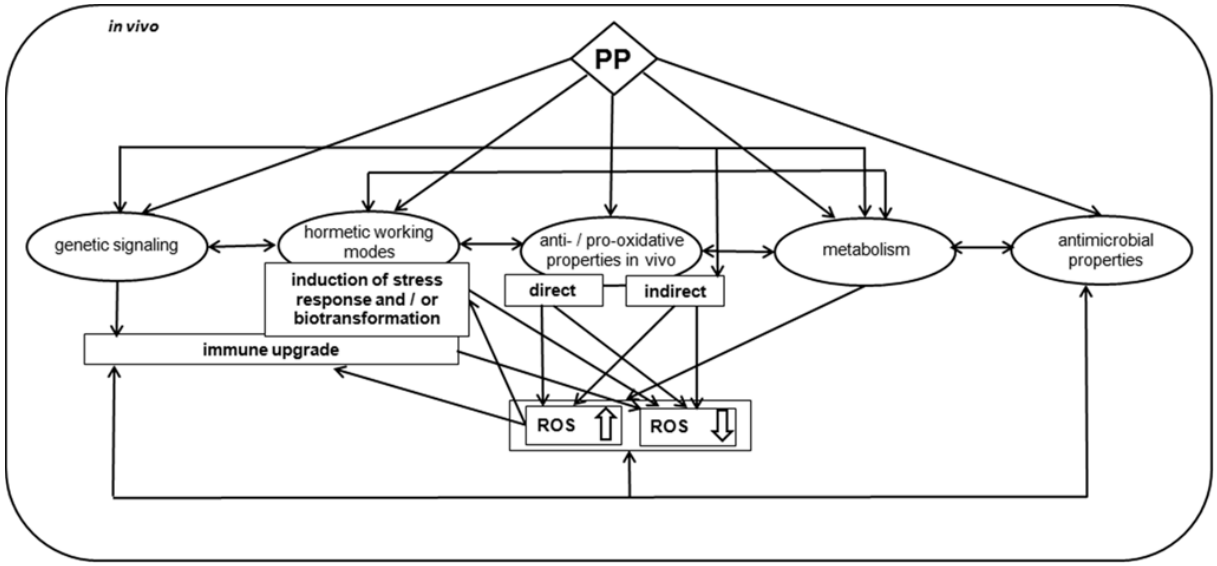


**Fig. 32 General working scheme for PPs *in vivo*.**

### 5.1.2 Outlook and future directions

This work showed that *C. elegans* can be used as a model system to define the impact PPs on a multicellular organism, and revealed underlying mechanisms. The next steps would be more detailed pharmacological analyses in *C. elegans* and confirmation of the findings in other model organisms and in humans. For example, the fact of altering the fat deposition and lipofuscin accumulation due to treatment with PPs could be initial point for pharmacological application of PPs in obesity, diabetes and neurodegenerative disorders. Sugaya et al. (2004) showed an increased lipofuscin accumulation in neurons, when experimentally induced diabetes in rats. Lowering the risk of diabetes by reducing the fat content, as well as lowering the lipofuscin accumulation in neurons by slowing down the aging process could reduce these age-associated diseases.

Furthermore, since there is a strong association between immune function and individual human lifespan (Ginaldi et al., 2001), the involvement of the p38 MAPK pathway in PPs action should be initial point for further research. The NSY-1/SEK-1/PMK-1 cassette is an evolutionari-

ly conserved module used in defense against pathogenic attack. Mammalian innate immunity uses molecular pathways conserved with flies and nematodes, even though these invertebrate animals lack classic adaptive immune systems. This innate immunity pathway appears to be commonly affected by multiple PPs including Q, blueberry polyphenols (Wilson et al., 2006), Tannic Acid (Saul et al., 2010), CA and RA in *C. elegans*. Such a generalized genetic mechanism is likely to be active in other organisms as well and may thus be used in medical issues to enhance pathogen resistance against infections such as pneumonia, influenza, nephritis and septicemia, which are among the ten top causes of death for people over 65 years old (Anderson and Smith, 2005).

Moreover does the involvement of *sir-2.1* in CA and RA action require further investigations: The acetylation and deacetylation of chromatin-interacting proteins is central to the epigenetic regulation of gene expression. Because of the emerging pathogenetic role of histone deacetylation in human disorders (like some cancers, reviewed in Glozak and Seto, 2007), a great deal of effort has been evolved to detect chemicals that modulate histone deacetylases activity (Johnstone, 2002; Grozinger and Schreiber, 2002). Howitz et al. (2003) showed that sirtuin activating compounds (STACs) like resveratrol and other small molecules can promote the survival of human cells and extend the replicative lifespan of yeast. It is likely that CA and RA also are capable to do so and therefore could be utilized as drugs.

Furthermore, PPs could be used as small-molecule modulators in biomedical research to investigate yet unknown points of interactions and combinatorial downstream effects of well known genetic pathways. It is becoming increasingly clear that conserved pathways, like the TGF-beta, ILS and p38 MAPK signaling, which all serve in adapting the organism to the environment, play prominent roles in at least some PP's mode of action. PPs activate and inhibit not just one single pathway, but rather provoke a complex interplay of various structures in different pathways, presumably dependent on their metabolic usability and bioavailability. PP treatments in *C. elegans* could be used to combinatorially activate such pathways and DNA-microarray analyses could provide insights of possible interactions. This may set the stage for rational design of RNA interference experiments, suitable mutant assay experiments and RT-PCR approaches as well as protein-based tests of genetic players.

In addition to defining the working modes of individual phytochemicals, we need to investigate how a mixture of various phytochemicals, as existent in natural food, influence effects of one another and thereby the health and aging of the consumer. This requires highly standardized test designs among different laboratories and comprehensive data storage in databases. Furthermore, integrative experimental design strategies including transcriptomics, proteomics and metabolomics platforms are called for to understand the systems-level properties of aging networks. This will in turn contribute to a better understanding of how nutrition affects organisms and processes which cause and counteract aging.

From an ecological perspective it would be interesting to investigate how population dynamics can be influenced by ingesting special types of food. Mathematical models could estimate how PP mediated alterations in lifespan of individuals affect the behavior of the population as a whole. Experiments with closely related species (e.g. *C. briggsae*) that occupy the same niches evaluate the species-specific susceptibility to certain PPs and calculate if they have the potential of triggering a micro-evolution.

### 5.1.3 Critical review

**Standardization of handling methods, test regimes and data evaluation**

Even though *C. elegans* is undoubtedly a suitable model to study complex circumstances *in vivo*, investigations about the impact of compounds on life parameters need to adhere some basical important issues (as recently reviewed in Gruber et al., 2009). It is becoming increasingly clear in the *C. elegans* research community that meticulously standardizing the handling methods and laboratory conditions (especially for lifespan experiments) is of utmost importance. Lifespan assays are very susceptible for minimal external (and presumably also internal) variations, making it difficult to generate robust lifespan data, as shown in single trial variations in this study, but also in the case of resveratrol in the literature. While Wood et al. (2004), Viswanathan et al. (2005) and Gruber et al. (2007) found lifespan extension due to exposure to resveratrol, Bass et al. (2007) could not confirm this finding. Within the framework of this study, it was also tried to reproduce results found for resveratrol in our laboratory, but we also could not find any life extension, rather a diminishment (data not shown). Sources for deviations are manifold: Little variations in room temperature, in the density and composition of the bacterial food (e.g. contaminations with other bacteria), the number of nematodes on the plates, the use of media and chemicals from differing firms and batches, as well as the humidity and thickness of the agar can provoke major discrepancies in results. In addition, genetic drift occurs when nematodes are maintained over many generations under laboratory conditions, if not regulatory refreshed from frozen stocks. Hence, resulting genetic differences can affect lifespan and other physiological traits, which sensitize worms to a given stimulus in aberrant extent. Differences in the numbers and the physical constrain of the transfer, and the use of hypochloride to synchronize worms can affect the status of the worms. Observer's bias can furthermore subconsciously influence the results. Therefore blinding of studies is necessary, but not always practicable due to colored compounds (like in this study). Moreover, there are issues about the handling of data, calculation of significances and the minimum sample sizes of an experiment, which all produce deviations in the results (for a very informative review be referred to Gruber et al., 2009).

Hence, every new starting *C. elegans* researcher should strongly be advised to inform about above discussed issues and thereafter prepare a fixed working scheme which has to be the basis of all following experiments. It should contain regimes for generating stocks in advance to large experiments and refreshing of populations, transfer of nematodes, growing bacteria and adjusting the same density, for pouring and storage of plates, as well as coding of blinded plates and evaluation and pooling of data. The laboratories should furthermore guarantee constant temperature, and have to be held close and clean to avoid contaminations, since the transfer of *C. elegans* cohorts in lifespan assays is time consuming and plates have to be handled outside thermo-cupboards and lamina flows.

**Interpretation of results**

Results from lifespan assays in mutant strains need to be interpreted with caution. For example *mev-1* mutants are usually used to determine antioxidative properties of compounds *in vivo*, due to their higher level of internal oxidative stress produced by the defective electron transport chain. A lack in lifespan prolongation due to a certain PP could be interpreted on one hand, as deficient antioxidant ability of the compound *in vivo*, but on the other, as *mev-1* being a genetic mediator of PP's longevity. The gain in life extension of *mev-1* mutants due to PPs treatment is usually explained with *in vivo* antioxidative properties of respective PP. In contrast, life extension in a *daf-16* mutant would be taken as evidence that *daf-16* can be excluded as a genetic

mediator. These variations in possibilities to interpret raise the question if lifespan assays with different mutant strains can be compared and interpreted without considering specifics of the underlying internal physiology. Furthermore it is conceivable that different genes are functionally redundant and therefore effects are masked when using a single mutant.

DNA-microarray studies allow to systematically measuring the genome-wide gene expression profile. However, an induction or repression of individual genes does not necessarily imply that they play a significant role in mediating a response. Moreover, other regulatory mechanisms (e.g. post-translational modifications) exist which modulate the activity of respective protein. Therefore it would have to be important to subsequently test on the protein level and furthermore on the metabolomics level to get deeper insights in the exact working patterns *in vivo*.

**Difficulty of identifying underlying working modes of compounds**

The present study revealed that only a broad spectrum of different tests can help to elucidate the *in vivo* actions of phytochemicals. For example, the *in vitro* antioxidant properties, are not necessarily associated to *in vivo* antioxidant properties (cf. Pun et al., 2010), and the *in vivo* antioxidant properties cannot always explain the full impact on the organism. Moreover, it has to be investigated to which extent direct or indirect CR effects, hormetic mechanisms and other events contribute to observed phenotypes.
Furthermore, this study has shown that simply evaluating DNA-microarrays with prevalent methods, like GO-term and KEGG pathway-analyses, is not always enough to understand underlying genetic mechanisms. Functional insights rather require detailed comparison with published DNA-microarray studies in *C. elegans*, especially investigations about the global transcriptional response of certain mutant strains. This example shows the growing need for comparative databases which evaluate the congruence of certain datasets and hence allows drawing conclusions on underlying genetic players beyond simple GO and KEGG adjustment.

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## Weblinks

http://bioinf.wehi.edu.au/software/russell/logrank/
http://david.abcc.ncifcrf.gov
http://en.wikipedia.org/wiki/Cysteine
http://en.wikipedia.org/wiki/List_of_phytochemicals_in_food
http://lpi.oregonstate.edu
http://www.genome.jp/dbget-bin/www_bget?map04142
http://www.genome.jp/dbget-bin/www_bget?map04142
http://www.gepas.org
http://www.programmed-aging.org
http://www.wormbase.org

Online Supplementary 1:
https://docs.google.com/leaf?id=0B-
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Online Supplementary 2:
https://docs.google.com/leaf?id=0B-
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authkey=CNuBhcgP

# Appendix

**Supplementary Table 1 List of Phytochemicals in food**

| | | | |
|---|---|---|---|
| **Phenolic compounds** | **Monophenols** | • Apiole<br>• Carnosol<br>• Carvacrol<br>• Dillapiole.<br>• Rosemarinol | • parsley<br>• rosemary<br>• oregano, thyme<br>• dill<br>• rosemary |
| | **Flavonoids (PPs)** | *Flavonols*<br>• Quercetin<br>• Gingerol<br>• Kaempferol<br>• Myricetin<br>• Rutin<br>• Isorhamnetin | • onions, tea, wine, apples, cranberries, buckwheat, beans<br>• ginger<br>• strawberries, gooseberries, cranberries, peas, brassicates, chives<br>• grapes, walnuts<br>• citrus fruits, buckwheat, parsley, tomato, apricot, rhubarb, tea |
| | | *Flavanones*<br>• Hesperidin<br>• Naringenin<br>• Silybin<br>• Eriodictyol | • citrus fruits<br>• citrus fruits<br>• blessed milk thistle |
| | | *Flavones*<br>• Apigenin<br>• Tangeritin<br>• Luteolin | • chamomile, celery, parsley<br>• tangerine and other citrus peels |
| | | *Flavan-3-ols*<br>• Catechins<br>• (+)-Catechin<br>• (+)-Gallocatechin<br>• (-)-Epicatechin<br>• (-)-Epigallocatechin<br>• (-)-Epigallocatechin gallate (EGCG)<br>• (-)-Epicatechin 3-gallate<br>• Theaflavin<br>• Theaflavin-3-gallate Theaflavin-3'-gallate<br>• Theaflavin-3,3'-digallate<br>• Thearubigins | • white tea, green tea, black tea, grapes, wine, apple juice, cocoa, lentils, black-eyed peas<br>• green tea<br>• black tea<br>• black tea<br>• black tea<br>• black tea |
| | | *Anthocyanins (flavonals) and Anthocyanidins*<br>• Pelargonidin<br>• Peonidin<br>• Cyanidin<br>• Delphinidin<br>• Malvidin<br>• Petunidin | e.g. red wine, many red, purple or blue fruits and vegetables<br>• bilberry, raspberry, strawberry<br>• bilberry, blueberry, cherry, cranberry, peach, red apple & pear, bilberry, blackberry, blueberry, cherry, cranberry, peach, plum, hawthorn, loganberry, cocoa<br>• bilberry, blueberry, eggplant<br>• bilberry, blueberry |
| | | *Isoflavones (phytoestrogens)*<br>• Daidzein (formononetin)<br>• Genistein (biochanin A)<br>• Glycitein | • soy, alfalfa sprouts, red clover,chickpeas, peanuts, other legumes<br>• soy, alfalfa sprouts, red clover, chickpeas, peanuts, other legumes<br>• soy |

**Table continues on page 139**

**Continuation Supplementary Table 1**

| | | | |
|---|---|---|---|
| | | *Dihydroflavonols* | |
| | | *Chalcones* | |
| | | *Coumestans (phytoestrogens)*<br>• Coumestrol | • red clover, alfalfa sprouts, soy, peas, brussels sprouts |
| | **Phenolic acids** | • Ellagic acid<br>• Gallic acid<br>• Salicylic acid<br>• Tannic acid<br>• Vanillin | • walnuts, strawberries, cranberries, blackberries, guava, grapes<br>• tea, mango, strawberries, rhubarb, soy<br>• peppermint, licorice, peanut, wheat<br>• nettles, tea, berries<br>• vanilla beans, cloves |
| | **Phenolic acids** | • Capsaicin<br>• Curcumin | • chilli peppers<br>• turmeric, mustard |
| | **Hydroxycinnamic acids** | • Caffeic acid<br>• Chlorogenic acid<br>• Cinnamic acid<br>• Ferulic acid<br>• Coumarin | • burdock, hawthorn, artichoke, pear, basil, thyme, oregano, apple<br>• echinacea, strawberries, pineapple, coffee, sunflower, blueberries<br>• aloe<br>• oats, rice, artichoke, orange, pineapple, apple, peanut<br>• citrus fruits, maize |
| | **Lignans (phytoestrogens)** | • Silymarin<br>• Matairesinol<br>• Secoisolariciresinol<br>• Pinoresinol and lariciresinol | • seeds (flax, sesame, pumpkin, sunflower, poppy), whole grains (rye, oats, barley), bran (wheat, oat, rye), fruits (particularly berries) and vegetables<br>• artichokes, milk thistle<br>• flax seed, sesame seed, rye bran and meal, oat bran, poppy seed, strawberries, blackcurrants, broccoli<br>• flax seeds, sunflower seeds, sesame seeds, pumpkin, strawberries, blueberries, cranberries, zucchini, blackcurrant, carrots<br>• sesame seed, Brassica vegetables |
| | **Tyrosol esters** | • Tyrosol<br>• Hydroxytyrosol<br>• Oleocanthal<br>• Oleuropein | • olive oil<br>• olive oil<br>• olive oil<br>• olive oil |
| | **Stilbenoids** | • Resveratrol<br>• Pterostilbene<br>• Piceatannol | • grape skins and seeds, wine, nuts, peanuts<br>• grapes, blueberries<br>• grapes |
| | **Punicalagins** | | • pomegranates |
| **Terpenes (isoprenoids)** | **Carotenoids (tetraterpenoids)** | *Carotenes* (orange pigments)<br>• α-Carotene<br>• β-Carotene<br>• γ-Carotene<br>• δ-Carotene<br>• Lycopene<br>• Neurosporene<br>• Phytofluene<br>• Phytoene | • in carrots, pumpkins, maize, tangerine, orange<br>• in dark, leafy greens and red, orange and yellow fruits and vegetables<br>• Vietnam Gac, tomatoes, grapefruit, watermelon, guava, apricots, carrots<br>• star fruit, sweet potato, orange<br>• sweet potato, orange. |

**Table continues on page 140**

**Continuation Supplementary Table 1**

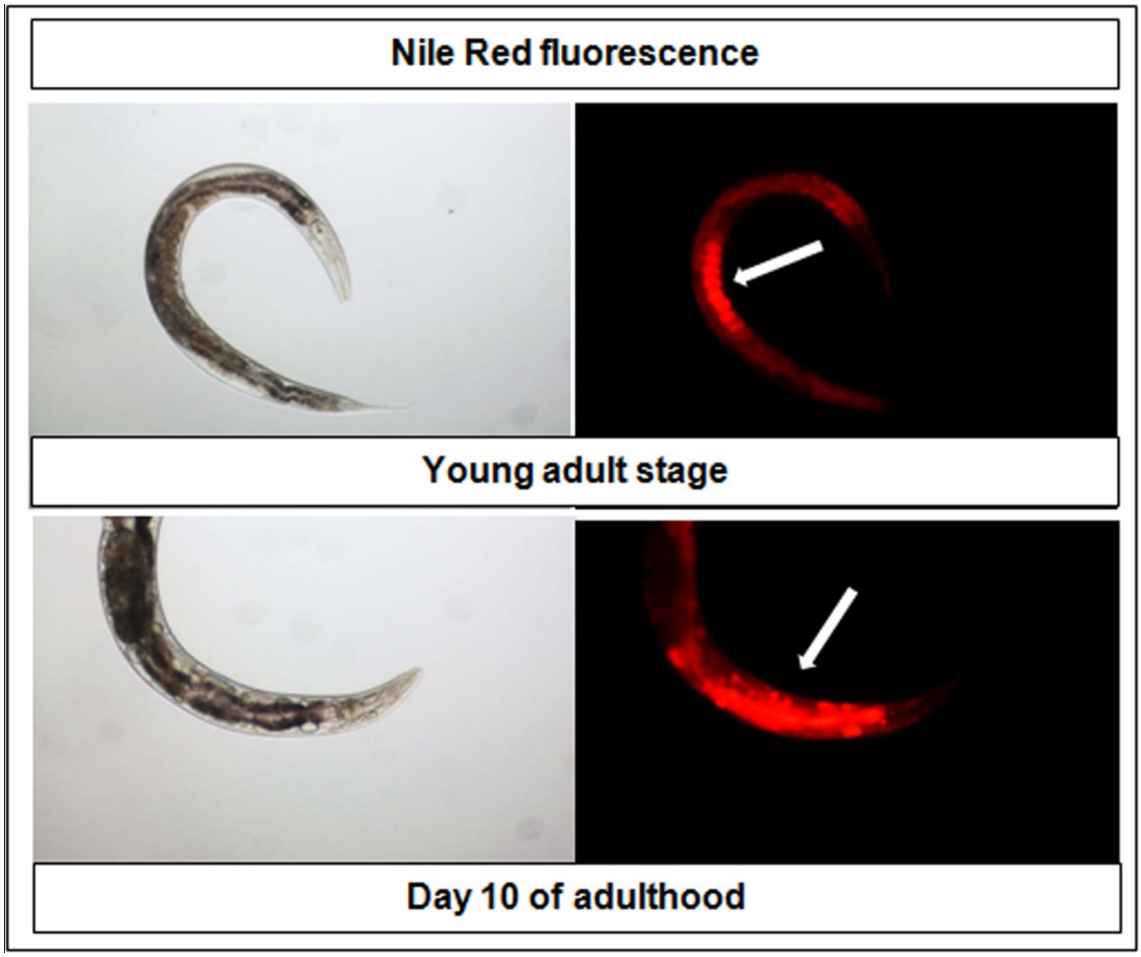
| | | | |
|---|---|---|---|
| **Terpenes (isoprenoids)** | **Carotenoids (tetraterpenoids)** | *Xanthophylls* (yellow pigments) | |
| | | • Canthaxanthin | • paprika |
| | | • Cryptoxanthin | • mango, tangerine, orange, papaya, peaches, avocado, pea, grapefruit, kiwi |
| | | • Zeaxanthin | • wolfberry, spinach, kale, turnip greens, maize, eggs, red pepper, pumpkin, oranges |
| | | • Astaxanthin | • microalge, yeast, krill, shrimp, salmon, lobsters, some crabs |
| | | • Lutein | • spinach, turnip greens, romaine lettuce, eggs, red pepper, pumpkin, mango, papaya, oranges, kiwi, peaches, squash, legumes, brassicates, prunes, sweet potatoes, honeydew melon, rhubarb, plum, avocado, pear |
| | | • Rubixanthin | • rose hips |
| | **Monoterpenes** | • Limonene | • oils of citrus, cherries, spearmint, dill, garlic, celery, maize, rosemary, ginger, basil |
| | | • Perillyl alcohol | • citrus oils, caraway, mints |
| | **Saponins** | | • soybeans, beans, other legumes, maize, alfalfa |
| | **Lipids** | *Phytosterols* | e.g. almonds, cashews, peanuts, sesame seeds, sunflower seeds, whole wheat, maize, soybeans, many vegetable oils |
| | | • Campesterol | • buckwheat |
| | | • beta Sitosterol | • avocados, rice bran, wheat germ, corn oils, fennel, peanuts, soybeans, hawthorn, basil, buckwheat |
| | | • gamma sitosterol | |
| | | • Stigmasterol | • buckwheat |
| | | *Tocopherols (vitamin E)* | |
| | **Lipids** | *omega-3,6,9 fatty acids* | e.g. dark-green leafy vegetables, grains, legumes, nuts |
| | | • gamma-linolenic acid | • evening primrose, borage, blackcurrant |
| | **Triterpenoid** | • Oleanolic acid | • American pokeweed, honey mesquite, garlic, java apple, cloves, and many other Syzygium species |
| | | • Ursolic acid | • apples, basil, bilberries, cranberries, elder flower, peppermint, lavender, oregano, thyme, hawthorn, prunes |
| | | • Betulinic acid | • Ber tree, white birch, tropical carnivorous plants *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, *Diospyrol leucomelas* a member of the persimmon family, *Tetracera boiviniana*, the jambul (*Syzygium formosanum*), and many other Syzygium species. |
| | | • Moronic acid | • Rhus javanica (a sumac), mistletoe |
| **Betalains** | **Betalains** | *Betacyanins* | |
| | | • betanin | • beets |
| | | • isobetanin | • beets |
| | | • probetanin | • beets |
| | | • neobetanin | • beets |
| | | *Betaxanthins* | |
| | | • Indicaxanthin | • beets, sicilian prickly pear |
| | | • Vulgaxanthin | • beets |
| **Organosulfides** | **Dithiolthiones (isothiocyanates)** | • Sulphoraphane | • Brassicates. |

**Table continues on page 141**

**Continuation Supplementary Table 1**

| | | | |
|---|---|---|---|
| | **Thiosulphonates (allium compounds)** | • Allyl methyl trisulfide<br>• Diallyl sulfide | • garlic, onions, leeks, chives, shallots<br>• garlic, onions, leeks, chives, shallots |
| **Indoles, glucosinolates** | | • Indole-3-carbinol<br>• sulforaphane<br>• 3,3'-Diindolylmethane or DIM<br>• Sinigrin<br>• Allicin<br>• Alliin<br>• Allyl isothiocyanate<br>• Piperine<br>• Syn-propanethial-S-oxide | • cabbage, kale, brussels sprouts, rutabaga, mustard greens<br>• broccoli family<br>• broccoli family<br>• broccoli family<br>• garlic<br>• garlic<br>• horseradish, mustard, wasabi<br>• black pepper<br>• onions |
| **Protein inhibitors** | | Protease inhibitors | • soy, seeds, legumes, potatoes, eggs, cereals |
| **Other organic acids** | | • Oxalic acid<br>• Phytic acid (inositol hexaphosphate)<br>• Tartaric acid<br>• Anacardic acid | • orange, spinach, rhubarb, tea and coffee, banana, ginger, almond, sweet potato, bell pepper<br>• cereals, nuts, sesame seeds, soybeans, wheat, pumpkin, beans, almonds<br>• apricots, apples, sunflower, avocado, grapes<br>• cashews, mangoes |

Source: From Wikipedia, the free encyclopedia
http://en.wikipedia.org/wiki/List_of_phytochemicals_in_food




**Supplementary Fig. 1 Nile Red staining of young adults and and 10 day old nematodes**: repeatedly found artifacts. In young adults seemingly the eggs also get stained by Nile Red what provoke falsifications; and in 10 days old adults obviously loose the intestine structure its integrity, what leads to overstaining. Since these events were observed frequently, the Nile Red measurements have been restricted to 3 and 6 days old adults.

**Supplementary Table 2 Overlapping and exclusively overrepresented GO-terms in single concentrations of Q, $Q_{longevity}$ and $Q_{all}$** (referes to Fig. 28)

| Q 50 µM | Q 100 µM | Q 200 µM | Q 50 & 100 µM | Q 50 & 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|
| **BIOLOGICAL PROCESS** | | | | | | |
| GO:0006082~organic acid metabolic process | GO:0005975~carbohydrate metabolic process | GO:0045449~regulation of transcription | x | GO:0006631~fatty acid metabolic process | GO:0007399~nervous system development | GO:0010171~body morphogenesis |
| GO:0043436~oxoacid metabolic process | GO:0040012~regulation of locomotion | GO:0045595~regulation of cell differentiation | | GO:0040002~collagen and cuticulin-based cuticle development | GO:0048699~generation of neurons | GO:0055114~oxidation reduction |
| GO:0019752~carboxylic acid metabolic process | GO:0007610~behavior | GO:0010556~regulation of macromolecule biosynthetic process | | | GO:0022008~neurogenesis | GO:0006323~DNA packaging |
| GO:0042180~cellular ketone metabolic process | GO:0022607~cellular component assembly | GO:0009889~regulation of biosynthetic process | | | GO:0007409~axonogenesis | GO:0065004~protein-DNA complex assembly |
| GO:0008152~metabolic process | GO:0045165~cell fate commitment | GO:0031326~regulation of cellular biosynthetic process | | | GO:0048667~cell morphogenesis involved in neuron differentiation | GO:0034728~nucleosome organization |
| GO:0006725~cellular aromatic compound metabolic process | GO:0043067~regulation of programmed cell death | GO:0051171~regulation of nitrogen compound metabolic process | | | GO:0009653~anatomical structure morphogenesis | GO:0031497~chromatin assembly |
| GO:0051186~cofactor metabolic process | GO:0010941~regulation of cell death | GO:0019219~regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | | | GO:0030182~neuron differentiation | GO:0006334~nucleosome assembly |
| GO:0009308~amine metabolic process | GO:0006665~sphingolipid metabolic process | GO:0045454~cell redox homeostasis | | **6** | GO:0000904~cell morphogenesis involved in differentiation | GO:0032787~monocarboxylic acid metabolic process |
| GO:0046394~carboxylic acid biosynthetic process | GO:0006643~membrane lipid metabolic process | GO:0006952~defense response | | | GO:0006928~cell motion | GO:0042335~cuticle development |
| GO:0016053~organic acid biosynthetic process | GO:0008340~determination of adult life span | GO:0031323~regulation of cellular metabolic process | | | GO:0048812~neuron projection morphogenesis | GO:0007592~protein-based cuticle development |
| GO:0006629~lipid metabolic process | GO:0007568~aging | GO:0010468~regulation of gene expression | | | GO:0048666~neuron development | |
| GO:0009309~amine biosynthetic process | GO:0010259~multicellular organismal aging | GO:0008078~mesodermal cell migration | | | GO:0007411~axon guidance | |
| GO:0006732~coenzyme metabolic process | GO:0043053~dauer entry | GO:0007509~mesoderm migration | | | GO:0031175~neuron projection development | |
| GO:0006519~cellular amino acid and derivative metabolic process | GO:0048598~embryonic morphogenesis | GO:0080090~regulation of primary metabolic process | | | GO:0030154~cell differentiation | |
| | | GO:0045944~positive regulation of transcription from RNA polymerase II promoter | | | GO:0016331~morphogenesis of embryonic epithelium | |

**Table continues on page 144**

**Continuation Supplementary Table 2**

| Q 50 µM | Q 100 µM | Q 200 µM | Q 50 & 100 µM | Q 50 & 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|
| **BIOLOGICAL PROCESS** | | | | | | |
| | | GO:0045941~positive regulation of transcription | | | GO:0048858~cell projection morphogenesis | |
| | | GO:0010628~positive regulation of gene expression | | | GO:0048869~cellular developmental process | |
| | | GO:0019725~cellular homeostasis | | | GO:0040011~locomotion | |
| | | GO:0040018~positive regulation of multicellular organism growth | | | GO:0048856~anatomical structure development | |
| | | GO:0060255~regulation of macromolecule metabolic process | | | GO:0016477~cell migration | |
| | | GO:0051704~multi-organism process | | | GO:0032990~cell part morphogenesis | |
| | | GO:0010975~regulation of neuron projection development | | | GO:0030030~cell projection organization | |
| | | GO:0050770~regulation of axonogenesis | | | GO:0006355~regulation of transcription, DNA-dependent | |
| | | GO:0010769~regulation of cell morphogenesis involved in differentiation | | | GO:0007155~cell adhesion | |
| | | GO:0042592~homeostatic process | | | GO:0022610~biological adhesion | |
| | | GO:0051173~positive regulation of nitrogen compound metabolic process | | | GO:0051252~regulation of RNA metabolic process | |
| | | GO:0031328~positive regulation of cellular biosynthetic process | | | GO:0007178~transmembrane receptor protein serine/threonine kinase signaling pathway | |
| | | GO:0010557~positive regulation of macromolecule biosynthetic process | | | GO:0007167~enzyme linked receptor protein signaling pathway | |
| | | GO:0009891~positive regulation of biosynthetic process | | | GO:0048870~cell motility | |
| | | GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | | | GO:0051674~localization of cell | |
| | | GO:0006633~fatty acid | | | GO:0065008~regulation of | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | biosynthetic process | | | biological quality | |
| | | GO:0051240~positive regulation of multicellular organismal process | | | GO:0048468~cell development | |
| | | | | | GO:0007179~transforming growth factor beta receptor signaling pathway | |
| | | | | | GO:0032989~cellular component morphogenesis | |
| | | | | | GO:0001764~neuron migration | |
| | | | | | GO:0000902~cell morphogenesis | |
| | | | | | GO:0042074~cell migration involved in gastrulation | |
| | | | | | GO:0001667~ameboidal cell migration | |
| | | | | | GO:0008361~regulation of cell size | |
| | | | | | GO:0040014~regulation of multicellular organism growth | |
| | | | | | GO:0035121~tail morphogenesis | |
| | | | | | GO:0051239~regulation of multicellular organismal process | |
| | | | | | GO:0006333~chromatin assembly or disassembly | |
| | | | | | GO:0031344~regulation of cell projection organization | |
| | | | | | GO:0045893~positive regulation of transcription, DNA-dependent | |
| | | | | | GO:0051254~positive regulation of RNA metabolic process | |
| | | | | | GO:0051960~regulation of nervous system development | |
| | | | | | GO:0045664~regulation of neuron differentiation | |

**Table continues on page 146**

**Continuation Supplementary Table 2**

| Q 50 µM | Q 100 µM | Q 200 µM | Q 50 & 100 µM | Q 50 & 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|
| **BIOLOGICAL PROCESS** | | | | | | |
| | | | | | GO:0031589~cell-substrate adhesion | |
| | | | | | GO:0050767~regulation of neurogenesis | |
| | | | | | GO:0007160~cell-matrix adhesion | |
| | | | | | GO:0060284~regulation of cell development | |
| | | | | | GO:0009886~post-embryonic morphogenesis | |
| | | | | | GO:0022603~regulation of anatomical structure mor-phogenesis | |
| | | | | | GO:0045138~tail tip mor-phogenesis | |
| | | | | | GO:0018991~oviposition | |
| | | | | | GO:0007517~muscle organ development | |
| | | | | | GO:0033057~reproductive behavior in a multicellular organism | |
| | | | | | GO:0032879~regulation of localization | |
| | | | | | GO:0050794~regulation of cellular process | |
| | | | | | GO:0051235~maintenance of location | |
| | | | | | GO:0019098~reproductive behavior | |
| | | | | | GO:0030334~regulation of cell migration | |
| | | | | | GO:0050896~response to stimulus | |
| | | | | | GO:0032535~regulation of cellular component size | |
| | | | | | GO:0051270~regulation of cell motion | |
| | | | | | GO:0016337~cell-cell adhesion | |
| | | | | | GO:0048522~positive regulation of cellular process | |

| MOLECULAR FUNCTION | | | | | | |
|---|---|---|---|---|---|---|
| GO:0019842~vitamin binding | GO:0005509~calcium ion binding | GO:0045735~nutrient reservoir activity | x | x | GO:0005515~protein binding | GO:0042302~structural constituent of cuticle |
| GO:0016627~oxidoreductase activity, acting on the CH-CH group of donors | GO:0005529~sugar binding | GO:0004707~MAP kinase activity | | | GO:0003700~transcription factor activity | GO:0005198~structural molecule activity |
| GO:0003824~catalytic activity | | | | | GO:0030528~transcription regulator activity | GO:0016491~oxidoreductase activity |
| GO:0048037~cofactor binding | | | | | GO:0043565~sequence-specific DNA binding | |
| GO:0016614~oxidoreductase activity, acting on CH-OH group of donors | | | | | GO:0045735~nutrient reservoir activity | |
| | | | | | GO:0003677~DNA binding | |
| | | | | | GO:0005488~binding | |
| | | | | | GO:0004497~monooxygenase activity | |
| | | | | | GO:0016705~oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | |
| | | | | | GO:0016860~intramolecular oxidoreductase activity | |
| | | | | | GO:0004707~MAP kinase activity | |
| | | | | | GO:0003993~acid phosphatase activity | |
| **CELLULAR COMPONENT** | | | | | | |
| x | GO:0005887~integral to plasma membrane | x | x | x | GO:0031143~pseudopodium | GO:0005856~cytoskeleton |
| | GO:0030054~cell junction | | | | GO:0044459~plasma membrane part | GO:0000786~nucleosome |
| | GO:0031226~intrinsic to plasma membrane | | | | GO:0005788~endoplasmic reticulum lumen | GO:0032993~protein-DNA complex |
| | GO:0005921~gap junction | | | | GO:0005886~plasma membrane | GO:0000785~chromatin |
| | GO:0043232~intracellular non-membrane-bounded organelle | | | | GO:0005576~extracellular region | |
| | GO:0043228~non-membrane-bounded organelle | | | | GO:0005783~endoplasmic reticulum | |

**Table continues on page 148**

**Continuation Supplementary Table 2**

| Q 50 µM | Q 100 µM | Q 200 µM | Q 50 & 100 µM | Q 50 & 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|
| **CELLULAR COMPONENT** | | | | | | |
| | GO:0042995~cell projection | | | | GO:0043229~intracellular organelle | |
| | | | | | GO:0043226~organelle | |
| | | | | | GO:0044421~extracellular region part | |
| | | | | | GO:0045111~intermediate filament cytoskeleton | |
| | | | | | GO:0005882~intermediate filament | |
| | | | | | GO:0044424~intracellular part | |
| | | | | | GO:0044427~chromosomal part | |
| | | | | | GO:0005911~cell-cell junction | |
| | | | | | GO:0043231~intracellular membrane-bounded orga-nelle | |
| | | | | | GO:0043227~membrane-bounded organelle | |
| | | | | | GO:0005634~nucleus | |

**Supplementary Table 3: GO Cluster analysis with significantly up-regulated transcripts in Q 100 and 200 µM.** DAVID, adjusted on "medium classification stringency", $p < 0.05$. Displayed is one significant GO-term per cluster.

| Category | Term | Process | p-value |
|---|---|---|---|
| **Q 100 µM up-regulated** | | | |
| **BP** | GO:0031497 | chromatin assembly | 1.84E-04 |
| | GO:0044255 | cellular lipid metabolic process | 5.37E-03 |
| | GO:0032787 | monocarboxylic acid metabolic process | 1.77E-03 |
| | GO:0009451 | RNA modification | 2.89E-02 |
| | GO:0008152 | metabolic process | 4.90E-02 |
| **MF** | GO:0016788 | hydrolase activity, acting on ester bonds | 1.57E-03 |
| | GO:0004497 | monooxygenase activity | 1.91E-03 |
| **CC** | GO:0000786 | nucleosome | 1.26E-04 |
| | GO:0016021 | integral to membrane | 3.47E-02 |
| | GO:0005856 | cytoskeleton | 6.25E-04 |
| **Q 200 µM up-regulated** | | | |
| **BP** | GO:0006629 | lipid metabolic process | 3.57E-05 |
| | GO:0030259 | lipid glycosylation | 5.67E-03 |
| | GO:0065004 | protein-DNA complex assembly | 5.40E-05 |
| | GO:0007592 | protein-based cuticle development | 2.07E-02 |
| | GO:0006633 | fatty acid biosynthetic process | 2.07E-03 |
| | GO:0008152 | metabolic process | 9.42E-06 |
| | GO:0006470 | protein amino acid dephosphorylation | 7.22E-03 |
| | GO:0006189 | 'de novo' IMP biosynthetic process | 4.11E-03 |
| **MF** | GO:0042578 | phosphoric ester hydrolase activity | 9.78E-05 |
| | GO:0004497 | monooxygenase activity | 1.77E-04 |
| | GO:0004091 | carboxylesterase activity | 1.75E-02 |
| | GO:0030246 | carbohydrate binding | 1.96E-02 |
| | GO:0016705 | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | 3.17E-02 |
| | GO:0008237 | metallopeptidase activity | 3.45E-02 |
| **CC** | GO:0000786 | nucleosome | 5.15E-05 |
| | GO:0005764 | lysosome | 9.07E-02 |
| | GO:0016021 | integral to membrane | 2.64E-02 |
| | GO:0005856 | cytoskeleton | 6.51E-03 |

**Supplementary Table 4: GO Cluster analysis with significantly down-regulated transcripts in Q 100 and 200 µM.** DAVID, adjusted on "medium classification stringency", $p < 0.05$. Displayed is one significant GO-term per cluster.

| Category | Term | Process | p-Value |
|---|---|---|---|
| **Q 100 µM down-regulated** | | | |
| **BP** | GO:0007399 | nervous system development | 3.11E-15 |
| | GO:0007155 | cell adhesion | 2.06E-08 |
| | GO:0006928 | cell motion | 7.73E-09 |
| | GO:0040012 | regulation of locomotion | 1.32E-04 |
| | GO:0009653 | anatomical structure morphogenesis | 5.72E-10 |
| | GO:0040014 | regulation of multicellular organism growth | 9.08E-04 |
| | GO:0050789 | regulation of biological process | 3.77E-05 |
| | GO:0007179 | transforming growth factor beta receptor signaling pathway | 6.79E-03 |
| | GO:0042074 | cell migration involved in gastrulation | 1.19E-03 |
| | GO:0045137 | development of primary sexual characteristics | 8.84E-03 |
| | GO:0033057 | reproductive behavior in a multicellular organism | 5.84E-05 |
| | GO:0014018 | neuroblast fate specification | 1.61E-02 |
| | GO:0048583 | regulation of response to stimulus | 3.79E-03 |
| | GO:0022603 | regulation of anatomical structure morphogenesis | 8.38E-03 |
| | GO:0043053 | dauer entry | 1.59E-02 |
| | GO:0018988 | molting cycle, protein-based cuticle | 3.20E-02 |
| | GO:0008361 | regulation of cell size | 3.83E-03 |
| | GO:0006355 | regulation of transcription, DNA-dependent | 2.67E-04 |
| | GO:0051252 | regulation of RNA metabolic process | 3.10E-04 |
| | GO:0006357 | regulation of transcription from RNA polymerase II promoter | 2.43E-02 |
| | GO:0009887 | organ morphogenesis | 9.71E-03 |
| | GO:0065008 | regulation of biological quality | 5.23E-03 |
| | GO:0045185 | maintenance of protein location | 2.77E-02 |
| | GO:0048646 | anatomical structure formation involved in morphogenesis | 1.63E-03 |
| | GO:0010646 | regulation of cell communication | 3.56E-02 |
| | GO:0042464 | dosage compensation, by hypoactivation of X chromosome | 2.51E-02 |
| | GO:0043067 | regulation of programmed cell death | 1.98E-02 |
| | GO:0048523 | negative regulation of cellular process | 1.97E-02 |
| | GO:0032787 | monocarboxylic acid metabolic process | 5.26E-02 |
| | GO:0007154 | cell communication | 2.87E-02 |
| | GO:0050793 | regulation of developmental process | 2.09E-02 |
| **MF** | GO:0030528 | transcription regulator activity | 2.73E-05 |
| | GO:0016862 | intramolecular oxidoreductase activity, interconverting keto- and enol-groups | 2.92E-02 |
| | GO:0016706 | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors | 2.36E-02 |
| **CC** | GO:0044459 | plasma membrane part | 2.64E-07 |
| | GO:0043231 | intracellular membrane-bounded organelle | 1.55E-04 |
| | GO:0030054 | cell junction | 5.04E-04 |
| | GO:0045111 | intermediate filament cytoskeleton | 4.59E-03 |
| | GO:0044421 | extracellular region part | 4.62E-03 |
| | GO:0005788 | endoplasmic reticulum lumen | 2.83E-04 |
| | GO:0005856 | cytoskeleton | 2.98E-02 |
| **Q 200 µM down-regulated** | | | |
| **BP** | GO:0007399 | nervous system development | 2.02E-14 |
| | GO:0007155 | cell adhesion | 5.61E-08 |
| | GO:0016477 | cell migration | 6.18E-07 |
| | GO:0040014 | regulation of multicellular organism growth | 1.06E-04 |
| | GO:0040012 | regulation of locomotion | 8.35E-04 |
| | GO:0033057 | reproductive behavior in a multicellular organism | 5.12E-05 |
| | GO:0009653 | anatomical structure morphogenesis | 3.44E-11 |
| | GO:0040008 | regulation of growth | 1.60E-03 |
| | GO:0007179 | transforming growth factor beta receptor signaling pathway | 8.39E-03 |
| | GO:0042074 | cell migration involved in gastrulation | 1.53E-04 |
| | GO:0006355 | regulation of transcription, DNA-dependent | 7.47E-07 |
| | GO:0048608 | reproductive structure development | 9.48E-03 |
| | GO:0065008 | regulation of biological quality | 1.69E-04 |
| | GO:0018988 | molting cycle, protein-based cuticle | 1.38E-02 |

**Table continues on page 151**

**Continuation of Supplementary Table 4**

| | | | |
|---|---|---|---|
| **BP** | GO:0008361 | regulation of cell size | 1.16E-03 |
| | GO:0045944 | positive regulation of transcription from RNA polymerase II promoter | 1.22E-02 |
| | GO:0014018 | neuroblast fate specification | 1.80E-02 |
| | GO:0048583 | regulation of response to stimulus | 6.11E-03 |
| | GO:0043053 | dauer entry | 2.08E-02 |
| | GO:0010646 | regulation of cell communication | 1.77E-02 |
| | GO:0048513 | organ development | 1.68E-02 |
| | GO:0045185 | maintenance of protein location | 3.36E-02 |
| | GO:0006631 | fatty acid metabolic process | 3.35E-03 |
| | GO:0042464 | dosage compensation, by hypoactivation of X chromosome | 2.94E-02 |
| | GO:0050793 | regulation of developmental process | 7.91E-03 |
| **MF** | GO:0030528 | transcription regulator activity | 1.09E-07 |
| | GO:0016860 | intramolecular oxidoreductase activity | 2.41E-02 |
| | GO:0016706 | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors | 2.70E-02 |
| **CC** | GO:0043231 | intracellular membrane-bounded organelle | 2.56E-05 |
| | GO:0005886 | plasma membrane | 1.07E-05 |
| | GO:0030054 | cell junction | 3.04E-03 |
| | GO:0045111 | intermediate filament cytoskeleton | 6.01E-03 |
| | GO:0044421 | extracellular region part | 2.36E-02 |
| | GO:0005788 | endoplasmic reticulum lumen | 4.03E-04 |
| | GO:0005856 | cytoskeleton | 3.61E-02 |

**Supplementary Table 5 Overrepresented KEGG pathways found in Q 100 µM treated nematodes.** Further summarized are the DEGs and statistical proven expression values (FC), p-values and the Fold Enrichment. The numbers in italics show FC values without guarantee of statistical robustness.

| Overrepresented KEGG pathways in Q 100 µM | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel04142: Lysosome** | | | | | | |
| 0.000 | 2.734 | *apm-3* | *0.86* | 0.75 | *0.65* | Clathrin-associated protein medium chain |
| | | *asm-2* | *1.20* | 1.38 | *1.79* | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | *asm-3* | *0.60* | 0.71 | *0.95* | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | C33G3.4 | *0.89* | 0.73 | *0.68* | Predicted beta-mannosidase |
| | | *cpr-2* | *1.56* | 5.83 | *8.28* | Cysteine proteinase Cathepsin L |
| | | *cpr-3* | *0.70* | 0.71 | *0.81* | cathepsin B-like cysteine proteinase |
| | | *cpr-3* | *0.70* | 0.64 | *0.73* | Cysteine proteinase Cathepsin L |
| | | F32H5.1 | *1.10* | 1.57 | *1.92* | Cysteine proteinase Cathepsin L |
| | | F57F5.1 | *1.07* | 0.78 | *0.86* | Cysteine proteinase Cathepsin L |
| | | R07E4.4 | *0.88* | 0.73 | *0.78* | Nucleoside phosphatase |
| | | *smf-3* | *0.79* | 1.30 | *1.16* | ST.LOUIS) |
| | | *vha-15* | *0.87* | 0.77 | *0.75* | Vacuolar H+-ATPase V1 sector, subunit H |
| | | W07B8.4 | *2.61* | 3.57 | *4.11* | thiol protease; C.elegans cDNA clone yk150b4 |
| | | Y16B4A.2 | *1.24* | 0.73 | *0.82* | Serine carboxypeptidases (lysosomal cathepsin A) |
| | | Y4C6B.6 | *1.42* | 1.30 | *1.57* | Beta-glucocerebrosidase |
| | | *nuc-1* | *0.83* | 0.83 | *0.94* | abnormal NUClease /// locus:nuc-1/Deoxyribonuclease II |
| | | *ncr-1* | *0.58* | 0.83 | *0.74* | Cholesterol transport protein (Niemann-Pick C disease protein) |
| | | Y40D12A.2 | *1.33* | 0.63 | *0.71* | Serine carboxypeptidases (lysosomal cathepsin A) |
| **cel04350: TGF-beta signaling pathway** | | | | | | |
| 0.001 | 3.551 | *daf-1* | *0.89* | 0.79 | *0.73* | C.elegans cDNA clone yk18f7 |
| | | *skr-10* | *0.99* | 0.68 | *0.56* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12* | *1.01* | 0.63 | *0.58* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | *1.03* | 0.72 | *0.62* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | *1.04* | 0.80 | *0.67* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | *0.95* | 0.76 | *0.65* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | *0.78* | 0.74 | *0.55* | RNA polymerase II elongation factor like; C.elegans cDNA clone yk98d5 |
| | | *skr-21* | *0.78* | 0.72 | *0.52* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | *1.02* | 0.68 | *0.61* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *0.98* | 0.71 | *0.57* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *1.00* | 0.69 | *0.57* | cyclin A-associated protein; C.elegans cDNA clone yk282d6 |
| | | *skr-9* | *0.95* | 0.67 | *0.56* | SCF ubiquitin ligase, Skp1 component |
| | | *skr-16* | *0.90* | 0.82 | *0.70* | SCF ubiquitin ligase, Skp1 component |
| **cel00600: Sphingolipid metabolism** | | | | | | |
| 0.001 | 4.648 | *asm-2* | *1.20* | 1.38 | *1.79* | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | *asm-3* | *0.60* | 0.71 | *0.95* | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | *sms-2* | *0.77* | 0.76 | *0.63* | Uncharacterized conserved protein |

**Table continues on page 153**

**Continuation Supplementary Table 5**

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *sptl-2* | 1.46 | 0.79 | 0.74 | serine palmtoyltransferase (ST.LOUIS) TR:Q20375 protein_id:AAA92303.1 |
| | | Y54E5A.1 | 0.88 | 0.78 | 0.80 | (HINXTON) |
| | | Y4C6B.6 | 1.42 | 1.30 | 1.57 | Beta-glucocerebrosidase |
| | | *spl-1* | 1.07 | 0.80 | 0.86 | Glutamate decarboxylase/sphingosine phosphate lyase |
| | | *sptl-3* | 1.28 | 0.85 | 0.93 | Serine palmitoyltransferase |
| **cel04310: Wnt signaling pathway** | | | | | | |
| 0.001 | 2.712 | *cwn-2* | 0.85 | 0.75 | 0.65 | Wnt family of developmental regulators |
| | | *dsh-1* | 1.01 | 0.71 | 0.65 | cDNA clone:yk653h6 |
| | | *dsh-1* | 0.75 | 0.69 | 0.61 | dishevelled-like protein |
| | | *jnk-1* | 0.71 | 0.74 | 0.62 | MAP kinase |
| | | *skr-10* | 0.99 | 0.68 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12* | 1.01 | 0.63 | 0.58 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | 1.03 | 0.72 | 0.62 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | 1.04 | 0.80 | 0.67 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | 0.95 | 0.76 | 0.65 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | 0.78 | 0.74 | 0.55 | RNA polymerase II elongation factor like; C.elegans cDNA clone yk98d5 |
| | | *skr-21* | 0.78 | 0.72 | 0.52 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | 1.02 | 0.68 | 0.61 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | 0.98 | 0.71 | 0.57 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | 1.00 | 0.69 | 0.57 | cyclin A-associated protein; C.elegans cDNA clone yk282d6 |
| | | *skr-9* | 0.95 | 0.67 | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *unc-43* | 0.74 | 0.72 | 0.82 | Ca2+/calmodulin-dependent protein kinase, EF-Hand protein superfamily |
| | | *skr-16* | 0.90 | 0.82 | 0.70 | SCF ubiquitin ligase, Skp1 component |
| **cel00980: Metabolism of xenobiotics by cytochrome P450** | | | | | | |
| 0.005 | 3.576 | *alh-5* | 0.73 | 1.95 | 2.28 | Aldehyde dehydrogenase |
| | | *gst-38* | 0.58 | 0.64 | 1.23 | Glutathione S-transferase |
| | | *gst-4* | 1.97 | 1.72 | 1.97 | Glutathione S-transferase |
| | | *gst-5* | 1.13 | 1.27 | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | 1.78 | 2.32 | 1.83 | Alcohol dehydrogenase, class V |
| | | F35H8.6 | 0.82 | 1.46 | 1.44 | UDP-glucuronosyl and UDP-glucosyl transferase |
| | | *ugt-50* | 1.43 | 0.78 | 0.99 | UDP-glucuronosyltransferase |
| | | *ugt-50* | 0.89 | 0.71 | 0.60 | wEST00903 Early embryo, Stratagene (cat. no. 937007) cDNA clone CEESC45 similar to cDNA clone cm10b6, mRNA sequence. |
| | | W01A11.1 | 1.23 | 1.26 | 1.25 | Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily) |
| **cel00982: Drug metabolism** | | | | | | |
| 0.005 | 3.576 | *alh-5* | 0.73 | 1.95 | 2.28 | Aldehyde dehydrogenase |
| | | *fmo-2* | 0.69 | 3.22 | 4.47 | Flavin-containing monooxygenase |
| | | *gst-38* | 0.58 | 0.64 | 1.23 | Glutathione S-transferase |
| | | *gst-4* | 1.97 | 1.72 | 1.97 | Glutathione S-transferase |
| | | *gst-5* | 1.13 | 1.27 | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | 1.78 | 2.32 | 1.83 | Alcohol dehydrogenase, class V |
| | | *ugt-58* | 0.82 | 1.46 | 1.44 | UDP-glucuronosyl and UDP-glucosyl transferase |
| | | *ugt-50* | 1.43 | 0.78 | 0.99 | UDP-glucuronosyltransferase |
| | | *ugt-50* | 0.89 | 0.71 | 0.60 | UDP-glucuronosyltransferase |

**Table continues on page 154**

**Continuation Supplementary Table 5**

| Overrepresented KEGG pathways in Q 100 µM | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel00330: Arginine and proline metabolism** | | | | | | |
| 0.011 | 3.099 | *alh-1* | 1.14 | 1.45 | 1.42 | Aldehyde dehydrogenase 2 |
| | | *C10C5.4* | 1.15 | 1.68 | 1.95 | Aminoacylase ACY1 and related metalloex-opeptidases |
| | | *T09B4.8* | 0.83 | 0.74 | 0.78 | aminotransferase |
| | | *dpy-18* | 1.13 | 0.70 | 0.63 | cDNA clone:yk548g10 |
| | | *dpy-18* | 1.05 | 0.67 | 0.61 | Prolyl 4-hydroxylase alpha subunit |
| | | *F44G3.2* | 1.53 | 2.18 | 1.89 | Creatine kinases |
| | | *F55G1.9* | 1.17 | 1.29 | 1.32 | Pyrroline-5-carboxylate reductase |
| | | *phy-2* | 1.33 | 0.71 | 0.71 | Prolyl 4-hydroxylase alpha subunit |
| | | *smd-1* | 2.31 | 0.67 | 0.59 | Genomic Cluster |
| | | *smd-1* | 2.50 | 0.76 | 0.58 | S-adenosylmethionine decarboxylase |
| | | T22H6.2 | 1.29 | 0.73 | 0.69 | 1-pyroline-5-carboxylate synthetase (HINXTON) SW:P54889 pro-tein_id:CAA90672.1 |
| **cel00270: Cysteine and methionine metabolism** | | | | | | |
| 0.033 | 3.201 | T09B4.8 | 0.83 | 0.74 | 0.78 | aminotransferase |
| | | C06E7.3 | 1.33 | 1.35 | 1.29 | S-adenosylmethionine synthetase |
| | | *ldh-1* | 1.14 | 0.78 | 0.70 | Lactate dehydrogenase |
| | | *smd-1* | 2.31 | 0.67 | 0.59 | Genomic Cluster |
| | | *smd-1* | 2.50 | 0.76 | 0.58 | S-adenosylmethionine decarboxylase |
| | | *tag-32* | 3.32 | 2.57 | 4.18 | S-adenosylmethionine synthetase |
| | | ZC373.1 | 0.67 | 0.63 | 0.64 | Cystathionine beta-synthase and related en-zymes |
| **cel00350: Tyrosine metabolism** | | | | | | |
| 0.047 | 3.510 | *alh-5* | 0.73 | 1.95 | 2.28 | Aldehyde dehydrogenase |
| | | T09B4.8 | 0.83 | 0.74 | 0.78 | aminotransferase |
| | | R119.3 | 0.90 | 1.28 | 1.23 | Reductases with broad range of substrate spe-cificities |
| | | *sodh-1* | 1.78 | 2.32 | 1.83 | Alcohol dehydrogenase. class V |
| | | *tbh-1* | 0.94 | 0.80 | 0.78 | Dopamine beta-monooxygenase |

| < 0.65 | < 0.8 | <...> | > 1.25 | > 2.5 |
|---|---|---|---|---|

**Supplementary Table 6 Overrepresented KEGG pathways found in Q 200 µM treated nematodes.** Further summarized are the DEGs and statistical proven expression values (FC), p-values and the Fold Enrichment. The numbers in italics show FC values without guarantee of statistical robustness.

| Overrepresented KEGG pathways in Q 200 µM | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel04350: TGF-beta signaling pathway** | | | | | | |
| 0.000 | 3.718 | *daf-1* | *0.89* | *0.79* | 0.73 | C. elegans cDNA clone yk18f7 |
| | | *skr-10* | *0.99* | *0.68* | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12* | *1.01* | *0.63* | 0.58 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | *1.03* | *0.72* | 0.62 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | *1.04* | *0.80* | 0.67 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | *0.95* | *0.76* | 0.65 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-16* | *0.90* | *0.82* | 0.70 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | *0.78* | *0.74* | 0.55 | RNA polymerase II elongation factor like; C,elegans cDNA clone yk98d5 |
| | | *skr-21* | *0.78* | *0.72* | 0.52 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-5* | *1.08* | *1.00* | 1.42 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | *1.02* | *0.68* | 0.61 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *0.98* | *0.71* | 0.57 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *1.00* | *0.69* | 0.57 | cyclin A-associated protein; C.elegans cDNA clone yk282d6 |
| | | *skr-9* | *0.95* | *0.67* | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-16* | *0.90* | *0.82* | 0.70 | SCF ubiquitin ligase, Skp1 component |
| **cel04310: Wnt signaling pathway** | | | | | | |
| 0.001 | 2.574 | *cwn-2* | *0.85* | *0.75* | 0.65 | Wnt family of developmental regulators |
| | | *dsh-1* | *1.01* | *0.71* | 0.65 | cDNA clone:yk653h6 |
| | | *dsh-1* | *0.75* | *0.69* | 0.61 | dishevelled-like protein |
| | | *jnk-1* | *0.71* | *0.74* | 0.62 | MAP kinase |
| | | *skr-10* | *0.99* | *0.68* | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-12* | *1.01* | *0.63* | 0.58 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-13* | *1.03* | *0.72* | 0.62 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-14* | *1.04* | *0.80* | 0.67 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-15* | *0.95* | *0.76* | 0.65 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-16* | *0.90* | *0.82* | 0.70 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-21* | *0.78* | *0.74* | 0.55 | RNA polymerase II elongation factor like; C,elegans cDNA clone yk98d5 |
| | | *skr-21* | *0.78* | *0.72* | 0.52 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-5* | *1.08* | *1.00* | 1.42 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-7* | *1.02* | *0.68* | 0.61 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *0.98* | *0.71* | 0.57 | SCF ubiquitin ligase, Skp1 component |
| | | *skr-8* | *1.00* | *0.69* | 0.57 | cyclin A-associated protein; C. elegans cDNA clone yk282d6 |
| | | *skr-9* | *0.95* | *0.67* | 0.56 | SCF ubiquitin ligase, Skp1 component |
| | | *unc-43* | *0.74* | *0.72* | 0.82 | Ca2+/calmodulin-dependent protein kinase, EF-Hand protein superfamily |

**Table continues on page 156**

**Continuations Supplementary Table 6**

| Overrepresented KEGG pathways in Q 200 µM | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 10 µM (Fd) | Q 100 µM (Fd) | Q 200 µM (FC) | Description |
| **cel00982: Drug metabolism** | | | | | | |
| 0.005 | 3.195 | *alh-5* | *0.73* | *1.95* | 2.28 | Aldehyde dehydrogenase |
| | | F20G2.2 | *1.24* | *0.86* | 0.79 | Predicted short chain-type dehydrogenase |
| | | *fmo-1* | *1.07* | *0.98* | 1.25 | Flavin-containing monooxygenase |
| | | *fmo-2* | *0.69* | *3.22* | 4.47 | Flavin-containing monooxygenase |
| | | *gst-10* | *1.29* | *0.99* | 1.28 | Glutathione S-transferase |
| | | *gst-4* | *1.97* | *1.72* | 1.97 | Glutathione S-transferase |
| | | *gst-5* | *1.13* | *1.27* | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | *1.78* | *2.32* | 1.83 | Alcohol dehydrogenase. class V |
| | | *ugt-50* | *0.89* | *0.71* | 0.60 | wEST00903 Early embryo. Stratagene (cat. no. 937007) cDNA clone CEESC45 similar to cDNA clone cm10b6. mRNA sequence. |
| **cel00480: Glutathione metabolism** | | | | | | |
| 0.015 | 2.647 | C11E4.1 | *1.52* | *1.32* | 1.51 | glutathione peroxidase |
| | | C11E4.2 | *0.54* | *0.84* | 0.77 | Glutathione peroxidase |
| | | F59B8.2 | *1.21* | *0.90* | **0.81** | NADP-dependent isocitrate dehydrogenase |
| | | *gst-10* | *1.29* | *0.99* | 1.28 | Glutathione S-transferase |
| | | *gst-36* | *1.06* | *0.85* | **0.85** | Glutathione S-transferase |
| | | *gst-4* | *1.97* | *1.72* | 1.97 | Glutathione S-transferase |
| | | *gst-5* | *1.13* | *1.27* | 1.53 | Glutathione S-transferase |
| | | M176.2 | *0.94* | *0.86* | **0.81** | cDNA clone:yk639e6 |
| | | *rnr-2* | *1.03* | *0.90* | **0.84** | Ribonucleotide reductase, beta subunit |
| **cel00330: Arginine and proline metabolism** | | | | | | |
| 0.020 | 2.745 | *alh-1* | *1.14* | *1.45* | 1.42 | Aldehyde dehydrogenase 2 |
| | | *alh-9* | *0.99* | *0.81* | 0.79 | Aldehyde dehydrogenase |
| | | *dpy-18* | *1.26* | *0.85* | 0.77 | prolyl 4-hydroxylase alpha subunit |
| | | *dpy-18* | *1.13* | *0.70* | 0.63 | cDNA clone:yk548g10 |
| | | *dpy-18* | *1.05* | *0.67* | 0.61 | Prolyl 4-hydroxylase alpha subunit |
| | | F44G3.2 | *1.53* | *2.18* | 1.89 | Creatine kinases |
| | | F55G1.9 | *1.17* | *1.29* | 1.32 | Pyrroline-5-carboxylate reductase |
| | | *phy-2* | *1.33* | *0.71* | 0.71 | Prolyl 4-hydroxylase alpha subunit |
| | | *smd-1* | *2.31* | *0.67* | 0.59 | Genomic Cluster |
| | | *smd-1* | *2.50* | *0.76* | 0.58 | S-adenosylmethionine decarboxylase |
| | | T22H6.2 | *1.29* | *0.73* | 0.69 | 1-pyroline-5-carboxylate synthetase (HINXTON) SW:P54889 pro-tein_id:CAA90672.1 |
| **cel04142: Lysosome** | | | | | | |
| 0.026 | 1.968 | *apm-3* | *0.86* | *0.75* | 0.65 | Clathrin-associated protein medium chain |
| | | *asm-2* | *1.20* | *1.38* | 1.79 | Acid sphingomyelinase and PHM5 phosphate metabolism protein |
| | | C33G3.4 | *0.89* | *0.73* | 0.68 | Predicted beta-mannosidase |
| | | *cpr-2* | *1.56* | *5.83* | 8.28 | Cysteine proteinase Cathepsin L |
| | | *cpr-3* | *0.70* | *0.64* | 0.73 | Cysteine proteinase Cathepsin L |
| | | F32H5.1 | *1.10* | *1.57* | 1.92 | Cysteine proteinase Cathepsin L |
| | | *mig-23* | *0.88* | *0.73* | 0.78 | Nucleoside phosphatase |
| | | *ncr-1* | *0.58* | *0.83* | 0.74 | Cholesterol transport protein (Niemann-Pick C disease protein) |

**Table continues on page 157**

**Continuation of Supplementary Table 6**

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *vha-15* | *0.87* | *0.77* | 0.75 | Vacuolar H+-ATPase V1 sector. subunit H |
| | | W07B8.4 | *2.61* | *3.57* | 4.11 | thiol protease; C.elegans cDNA clone yk150b4 |
| | | Y4C6B.6 | *1.42* | *1.30* | 1.57 | Beta-glucocerebrosidase |
| | | Y40D12A.2 | *1.33* | *0.63* | 0.71 | Serine carboxypeptidases (lysosomal cathepsin A) |
| | | M05B5.4 | *0.85* | *0.87* | **0.81** | Lecithin:cholesterol acyltransferase (LCAT)/Acyl-ceramide synthase |
| | | Y16B4A.2 | *1.24* | *0.73* | **0.82** | Serine carboxypeptidases (lysosomal cathepsin A) |
| **cel00980: Metabolism of xenobiotics by cytochrome P450** | | | | | | |
| 0.033 | 2.772 | *alh-5* | *0.73* | *1.95* | 2.28 | Aldehyde dehydrogenase |
| | | F20G2.2 | *1.24* | *0.86* | 0.79 | Predicted short chain-type dehydrogenase |
| | | *gst-10* | *1.29* | *0.99* | 1.28 | Glutathione S-transferase |
| | | *gst-4* | *1.97* | *1.72* | 1.97 | Glutathione S-transferase |
| | | *gst-5* | *1.13* | *1.27* | 1.53 | Glutathione S-transferase |
| | | *sodh-1* | *1.78* | *2.32* | 1.83 | Alcohol dehydrogenase. class V |
| | | *ugt-58* | *0.82* | *1.46* | 1.44 | UDP-glucuronosyl and UDP-glucosyl transfe-rase |
| | | *ugt-50* | *0.89* | *0.71* | 0.60 | wEST00903 Early embryo. Stratagene (cat. no. 937007) cDNA clone CEESC45 similar to cDNA clone cm10b6. mRNA sequence. |
| | | *alh-1* | *1.14* | *1.45* | 1.42 | Aldehyde dehydrogenase 2 |
| **cel00340: Histidine metabolism** | | | | | | |
| 0.048 | 4.576 | *alh-5* | *0.73* | *1.95* | 2.28 | Aldehyde dehydrogenase |
| | | *alh-9* | *0.99* | *0.81* | 0.79 | Aldehyde dehydrogenase |
| | | Y51H4A.7 | *0.98* | *1.10* | 1.28 | Urocanate hydratase |

| < 0.65 | < 0.8 | <...> | > 1.25 | > 2.5 |
|---|---|---|---|---|

**Supplementary Table 7 Overrepresented KEGG pathway found in $Q_{all}$.** Further summarized are the DEGs and statistical proven expression values (FC), p-values and the Fold Enrichment. The numbers in italics show FC values without guarantee of statistical robustness.

| Overrepresented KEGG pathways in $Q_{all}$ | | | | | | |
|---|---|---|---|---|---|---|
| p-value | Fold Enrichment | CGC | Q 50 µM (FC) | Q 100 µM (FC) | Q 200 µM (FC) | Description |
| **cel00330: Arginine and proline metabolism** | | | | | | |
| 0.005 | 10.765 | F44G3.2 | 1.53 | 2.18 | 1.89 | Creatine kinases |
| | | *phy-2* | 1.33 | 0.71 | 0.71 | Prolyl 4-hydroxylase alpha subunit |
| | | *smd-1* | 2.50 | 0.76 | 0.58 | S-adenosylmethionine decarboxylase |
| | | T22H6.2a | 1.29 | 0.73 | 0.69 | 1-pyroline-5-carboxylate synthetase |

| < 0.65 | < 0.8 | <...> | > 1.25 | > 2.5 |
|---|---|---|---|---|

**Supplementary Table 8 DEGs and RFs in certain gene-groups and -classes** in Q 50, 100 and 200 µM treated nematodes and commonly regulated DEGs in pooled datasets of $Q_{longevity}$ and $Q_{all}$. Red numbers highlight significant RF values ($p < 0.05$). Refers to Table 17.

| Gene-classes and -groups | No. in class group | Q 50 µM | | | Q 100 µM | | | Q 200 µM | | | $Q_{longevity}$ | | | $Q_{all}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| ***abus/pqns*** | 117 | 4 | 1.06 | 1.98E-01 | 10 | 0.80 | 9.85E-02 | 12 | 0.84 | 9.72E-02 | 10 | 0.87 | 1.18E-01 | 2 | 0.87 | 2.68E-01 |
| **acetyltransferases** | 36 | 0 | 0.00 | 3.06E-01 | 2 | 0.52 | 1.56E-01 | 1 | 0.23 | 4.60E-02 | 1 | 0.28 | 9.60E-02 | 0 | 0.00 | 4.88E-01 |
| **acyltransferases** | 123 | 4 | 1.01 | 1.99E-01 | 13 | 0.99 | 1.17E-01 | 18 | 1.20 | 7.37E-02 | 11 | 0.91 | 1.19E-01 | 2 | 0.83 | 2.63E-01 |
| **amino acid metabolism** | 104 | 10 | 2.98 | 1.43E-03 | 17 | 1.54 | 2.19E-02 | 20 | 1.58 | 1.22E-02 | 16 | 1.57 | 2.15E-02 | 5 | 2.44 | 3.79E-02 |
| **aminoxidase** | 8 | 0 | 0.00 | 7.69E-01 | 2 | 2.35 | 1.61E-01 | 3 | 3.07 | 5.31E-02 | 2 | 2.56 | 1.44E-01 | 0 | 0.00 | 8.53E-01 |
| **biosynthese** | 441 | 29 | 2.04 | 1.44E-04 | 59 | 1.26 | 1.06E-02 | 65 | 1.21 | 1.51E-02 | 47 | 1.09 | 5.13E-02 | 9 | 1.04 | 1.34E-01 |
| **cell adhesion** | 45 | 6 | 4.13 | 2.54E-03 | 14 | 2.93 | 1.18E-04 | 14 | 2.55 | 4.74E-04 | 14 | 3.18 | 4.89E-05 | 4 | 4.51 | 9.86E-03 |
| **cell structure** | 201 | 37 | 5.70 | 0.00E+00 | 67 | 3.14 | 0.00E+00 | 70 | 2.85 | 0.00E+00 | 64 | 3.26 | 0.00E+00 | 31 | 7.83 | 0.00E+00 |
| **chromatin** | 14 | 0 | 0.00 | 6.31E-01 | 3 | 2.02 | 1.27E-01 | 2 | 1.17 | 2.84E-01 | 2 | 1.46 | 2.53E-01 | 0 | 0.00 | 7.57E-01 |
| **collagen** | 179 | 36 | 6.22 | 0.00E+00 | 46 | 2.42 | 6.07E-09 | 59 | 2.70 | 1.84E-13 | 46 | 2.63 | 4.36E-10 | 21 | 5.95 | 5.28E-11 |
| ***cyps*** | 81 | 5 | 1.91 | 7.43E-02 | 16 | 1.86 | 5.94E-03 | 18 | 1.82 | 4.48E-03 | 15 | 1.89 | 6.45E-03 | 5 | 3.13 | 1.66E-02 |
| ***dafs*** | 38 | 1 | 0.81 | 3.65E-01 | 4 | 0.99 | 2.07E-01 | 7 | 1.51 | 9.01E-02 | 4 | 1.08 | 2.04E-01 | 0 | 0.00 | 4.69E-01 |
| **decarboxylases** | 24 | 1 | 1.29 | 3.65E-01 | 5 | 1.96 | 6.82E-02 | 3 | 1.02 | 2.39E-01 | 3 | 1.28 | 2.18E-01 | 1 | 2.11 | 3.00E-01 |
| **dehydrogenases** | 151 | 15 | 3.07 | 8.39E-05 | 21 | 1.31 | 4.25E-02 | 25 | 1.36 | 2.57E-02 | 15 | 1.02 | 1.08E-01 | 3 | 1.01 | 2.27E-01 |
| **desaturases** | 10 | 0 | 0.00 | 7.20E-01 | 1 | 0.94 | 3.87E-01 | 2 | 1.64 | 2.37E-01 | 1 | 1.02 | 3.87E-01 | 0 | 0.00 | 8.20E-01 |
| **DNS repair** | 37 | 0 | 0.00 | 2.96E-01 | 3 | 0.76 | 2.05E-01 | 2 | 0.44 | 1.04E-01 | 2 | 0.55 | 1.74E-01 | 0 | 0.00 | 4.79E-01 |
| **DNS synthese** | 88 | 0 | 0.00 | 5.52E-02 | 0 | 0.00 | 4.94E-05 | 1 | 0.09 | 1.26E-04 | 0 | 0.00 | 1.15E-04 | 0 | 0.00 | 1.73E-01 |
| **energy generation** | 100 | 5 | 1.55 | 1.17E-01 | 12 | 1.13 | 1.11E-01 | 14 | 1.15 | 9.92E-02 | 9 | 0.92 | 1.34E-01 | 2 | 1.01 | 2.74E-01 |
| **esterases** | 100 | 5 | 1.55 | 1.17E-01 | 12 | 1.13 | 1.11E-01 | 16 | 1.31 | 5.83E-02 | 11 | 1.13 | 1.17E-01 | 2 | 1.01 | 2.74E-01 |
| **fatty acid oxidation** | 48 | 8 | 5.16 | 1.17E-04 | 4 | 0.78 | 1.77E-01 | 4 | 0.68 | 1.41E-01 | 1 | 0.21 | 3.72E-02 | 0 | 0.00 | 3.84E-01 |
| **glycolysis** | 7 | 0 | 0.00 | 7.94E-01 | 0 | 0.00 | 4.55E-01 | 0 | 0.00 | 4.02E-01 | 0 | 0.00 | 4.87E-01 | 0 | 0.00 | 8.70E-01 |
| **G-Protein coupled receptor** | 790 | 3 | 0.12 | 1.06E-08 | 11 | 0.13 | 0.00E+00 | 12 | 0.12 | 0.00E+00 | 10 | 0.13 | 0.00E+00 | 3 | 0.19 | 7.96E-05 |
| ***gsts*** | 58 | 7 | 3.74 | 2.03E-03 | 13 | 2.11 | 4.41E-03 | 14 | 1.98 | 5.34E-03 | 10 | 1.76 | 2.97E-02 | 1 | 0.87 | 3.68E-01 |
| **hermaphrodite enriched** | 100 | 1 | 0.31 | 1.24E-01 | 5 | 0.47 | 2.34E-02 | 9 | 0.74 | 8.19E-02 | 5 | 0.51 | 3.81E-02 | 1 | 0.51 | 2.75E-01 |
| **histone genes** | 57 | 7 | 3.80 | 1.85E-03 | 14 | 2.31 | 1.42E-03 | 16 | 2.30 | 6.66E-04 | 13 | 2.33 | 1.95E-03 | 6 | 5.34 | 7.52E-04 |
| **hsps/chaperones/alphy crystallins** | 37 | 1 | 0.84 | 3.67E-01 | 5 | 1.27 | 1.62E-01 | 8 | 1.77 | 4.36E-02 | 5 | 1.38 | 1.45E-01 | 0 | 0.00 | 4.79E-01 |
| **hydrolases** | 96 | 4 | 1.29 | 1.77E-01 | 16 | 1.57 | 2.18E-02 | 12 | 1.02 | 1.22E-01 | 12 | 1.28 | 8.42E-02 | 2 | 1.06 | 2.73E-01 |
| **insulin-related** | 25 | 1 | 1.24 | 3.68E-01 | 2 | 0.75 | 2.56E-01 | 4 | 1.31 | 1.83E-01 | 2 | 0.82 | 2.69E-01 | 0 | 0.00 | 6.08E-01 |
| **intestine** | 8 | 0 | 0.00 | 7.69E-01 | 1 | 1.18 | 3.87E-01 | 1 | 1.02 | 3.93E-01 | 0 | 0.00 | 4.39E-01 | 0 | 0.00 | 8.53E-01 |
| **kinases** | 579 | 9 | 0.48 | 5.01E-03 | 26 | 0.42 | 3.33E-08 | 41 | 0.58 | 1.43E-05 | 26 | 0.46 | 7.85E-07 | 7 | 0.61 | 5.38E-02 |
| **lectins** | 274 | 8 | 0.90 | 1.36E-01 | 36 | 1.24 | 3.03E-02 | 37 | 1.11 | 5.74E-02 | 31 | 1.16 | 5.36E-02 | 5 | 0.93 | 1.76E-01 |
| **lipid metabolism** | 288 | 26 | 2.79 | 1.76E-06 | 53 | 1.73 | 2.41E-05 | 58 | 1.65 | 3.68E-05 | 41 | 1.46 | 3.60E-03 | 11 | 1.94 | 1.60E-02 |
| **lysozyme** | 11 | 4 | 11.25 | 2.84E-04 | 5 | 4.28 | 3.19E-03 | 3 | 2.23 | 1.06E-01 | 3 | 2.79 | 6.77E-02 | 3 | 13.84 | 1.07E-03 |
| **male enriched** | 1404 | 90 | 1.98 | 1.45E-10 | 224 | 1.50 | 5.59E-11 | 266 | 1.55 | 1.34E-14 | 211 | 1.54 | 2.58E-11 | 78 | 2.82 | 0.00E+00 |
| **meiosis** | 22 | 0 | 0.00 | 4.85E-01 | 2 | 0.86 | 2.76E-01 | 1 | 0.37 | 1.74E-01 | 1 | 0.46 | 2.48E-01 | 0 | 0.00 | 6.45E-01 |
| **methyltransferases** | 65 | 3 | 1.43 | 1.93E-01 | 8 | 1.16 | 1.36E-01 | 7 | 0.88 | 1.48E-01 | 6 | 0.94 | 1.67E-01 | 1 | 0.78 | 3.59E-01 |
| **mitochondrial genes** | 152 | 5 | 1.02 | 1.79E-01 | 14 | 0.87 | 9.42E-02 | 18 | 0.97 | 9.91E-02 | 12 | 0.81 | 8.62E-02 | 3 | 1.00 | 2.27E-01 |
| **mitosis** | 69 | 0 | 0.00 | 1.03E-01 | 3 | 0.41 | 3.76E-02 | 4 | 0.47 | 4.04E-02 | 3 | 0.44 | 5.49E-02 | 0 | 0.00 | 2.53E-01 |
| **monooxygenases** | 15 | 0 | 0.00 | 6.11E-01 | 3 | 1.88 | 1.42E-01 | 5 | 2.73 | 2.21E-02 | 3 | 2.05 | 1.24E-01 | 0 | 0.00 | 7.42E-01 |
| ***msps*** | 67 | 28 | 12.93 | 0.00E+00 | 43 | 6.04 | 0.00E+00 | 44 | 5.38 | 0.00E+00 | 43 | 6.56 | 0.00E+00 | 28 | 21.21 | 0.00E+00 |
| **muscle** | 34 | 0 | 0.00 | 3.27E-01 | 4 | 1.11 | 2.04E-01 | 4 | 0.96 | 2.07E-01 | 3 | 0.90 | 2.31E-01 | 0 | 0.00 | 5.08E-01 |
| **neuronal genes** | 77 | 1 | 0.40 | 2.05E-01 | 7 | 0.86 | 1.42E-01 | 8 | 0.85 | 1.30E-01 | 6 | 0.80 | 1.39E-01 | 0 | 0.00 | 2.15E-01 |

**Table continues on page 159**

**Continuation Supplementary Table 8**

| Gene -classes and -groups | No. in class | Q 50 µM | | | Q 100 µM | | | Q 200 µM | | | $Q_{longevity}$ | | | $Q_{all}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| **nucleotid synthese** | 56 | 2 | 1.11 | 2.73E-01 | 5 | 0.84 | 1.68E-01 | 7 | 1.02 | 1.59E-01 | 4 | 0.73 | 1.59E-01 | 0 | 0.00 | 3.28E-01 |
| **oocyte enriched** | 252 | 0 | 0.00 | 2.41E-04 | 26 | 0.97 | 8.18E-02 | 32 | 1.04 | 7.37E-02 | 26 | 1.06 | 7.95E-02 | 0 | 0.00 | 6.42E-03 |
| **pdz** | 46 | 2 | 1.35 | 2.55E-01 | 9 | 1.84 | 2.98E-02 | 9 | 1.60 | 5.36E-02 | 9 | 2.00 | 1.99E-02 | 2 | 2.21 | 1.68E-01 |
| **peroxidases** | 18 | 3 | 5.16 | 1.68E-02 | 4 | 2.09 | 8.10E-02 | 5 | 2.28 | 4.27E-02 | 4 | 2.27 | 6.62E-02 | 1 | 2.82 | 2.53E-01 |
| **phosphatases** | 257 | 11 | 1.32 | 8.11E-02 | 28 | 1.02 | 7.94E-02 | 41 | 1.31 | 1.42E-02 | 27 | 1.07 | 7.55E-02 | 9 | 1.78 | 3.73E-02 |
| **programmed cell death** | 10 | 0 | 0.00 | 7.20E-01 | 2 | 1.88 | 2.07E-01 | 1 | 0.82 | 3.78E-01 | 1 | 1.02 | 3.87E-01 | 0 | 0.00 | 8.20E-01 |
| **proteases** | 113 | 7 | 1.92 | 4.35E-02 | 19 | 1.58 | 1.38E-02 | 20 | 1.45 | 2.35E-02 | 16 | 1.45 | 3.57E-02 | 5 | 2.25 | 4.84E-02 |
| ***sods*** | 5 | 1 | 6.19 | 1.42E-01 | 2 | 3.76 | 8.07E-02 | 2 | 3.28 | 1.01E-01 | 2 | 4.09 | 7.02E-02 | 1 | 10.15 | 9.10E-02 |
| **sperm-enriched** | 633 | 58 | 2.84 | 5.82E-13 | 99 | 1.47 | 2.02E-05 | 130 | 1.68 | 5.14E-10 | 98 | 1.58 | 1.23E-06 | 56 | 4.49 | 0.00E+00 |
| **sulfotransferases** | 29 | 0 | 0.00 | 3.86E-01 | 3 | 0.97 | 2.36E-01 | 3 | 0.85 | 2.25E-01 | 3 | 1.06 | 2.36E-01 | 0 | 0.00 | 5.61E-01 |
| **TF** | 298 | 9 | 0.93 | 1.32E-01 | 44 | 1.39 | 5.59E-03 | 55 | 1.51 | 4.59E-04 | 44 | 1.51 | 1.53E-03 | 7 | 1.19 | 1.40E-01 |
| **Ubiquitin genes** | 218 | 7 | 0.99 | 1.52E-01 | 21 | 0.91 | 8.14E-02 | 24 | 0.90 | 7.43E-02 | 18 | 0.84 | 7.25E-02 | 3 | 0.70 | 1.81E-01 |
| ***ugts*** | 43 | 2 | 1.44 | 2.46E-01 | 9 | 1.97 | 2.14E-02 | 8 | 1.52 | 7.51E-02 | 8 | 1.90 | 3.30E-02 | 1 | 1.18 | 3.68E-01 |
| **vitellogenin** | 11 | 0 | 0.00 | 6.97E-01 | 6 | 5.60 | 3.78E-04 | 8 | 5.96 | 5.46E-06 | 6 | 5.58 | 2.40E-04 | 0 | 0.00 | 8.03E-01 |

**Supplementary Table 9 Gene expression mountains.** Evaluation of global transcriptional output of Q 50, 100 and 200 µM treated nematodes, as well as commonly regulated DEGs in $Q_{longevity}$ and $Q_{all}$. Red numbers indicate significant ($p < 0.05$) overrepresentation ($RF > 1$). Refers to Table 19.

| mount | Q 50 µM | | | | Q 100 µM | | | | Q 200 µM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | up | | down | | up | | | | up | | down | |
| | RF | p | RF | | RF | p | RF | | RF | p | RF | |
| 0 | 0.15 | 0.00E+00 | 0.06 | 3.40E-07 | 0.13 | 0.00E+00 | 0.19 | 0.00E+00 | 0.17 | 0.00E+00 | 0.22 | 0.00E+00 |
| 1 | 0.24 | 5.65E-08 | 2.03 | 8.60E-04 | 0.31 | 5.94E-09 | 2.08 | 0.00E+00 | 0.33 | 1.03E-10 | 2.05 | 0.00E+00 |
| 2 | 0.24 | 4.09E-07 | 0.11 | 8.30E-04 | 0.48 | 4.26E-05 | 0.24 | 0.00E+00 | 0.35 | 8.70E-09 | 0.23 | 0.00E+00 |
| 3 | 0.18 | 5.87E-08 | 0.23 | 5.67E-03 | 0.18 | 3.99E-11 | 0.23 | 0.00E+00 | 0.18 | 6.17E-14 | 0.24 | 0.00E+00 |
| 4 | 3.83 | 0.00E+00 | 0.13 | 2.44E-03 | 4.41 | 0.00E+00 | 0.05 | 0.00E+00 | 4.40 | 0.00E+00 | 0.05 | 0.00E+00 |
| 5 | 0.28 | 1.75E-04 | 0.89 | 1.73E-01 | 0.12 | 7.00E-09 | 1.08 | 4.69E-02 | 0.15 | 4.13E-10 | 1.12 | 3.34E-02 |
| 6 | 0.84 | 7.69E-02 | 2.19 | 4.19E-03 | 1.45 | 4.49E-03 | 0.80 | 1.74E-02 | 1.61 | 1.13E-04 | 0.79 | 1.30E-02 |
| 7 | 0.12 | 3.72E-06 | 0.19 | 2.42E-02 | 0.53 | 3.31E-03 | 0.26 | 1.73E-10 | 0.41 | 6.23E-05 | 0.45 | 1.44E-06 |
| 8 | 3.00 | 1.21E-12 | 2.03 | 1.19E-02 | 3.71 | 0.00E+00 | 1.24 | 1.17E-02 | 3.68 | 0.00E+00 | 1.08 | 4.71E-02 |
| 9 | 0.44 | 2.85E-03 | 0.52 | 1.00E-01 | 0.57 | 4.51E-03 | 0.53 | 3.54E-05 | 0.47 | 1.82E-04 | 0.60 | 1.72E-04 |
| 10 | 0.00 | 2.23E-06 | 0.76 | 1.99E-01 | 0.00 | 6.40E-09 | 0.27 | 6.50E-08 | 0.00 | 2.78E-11 | 0.25 | 5.95E-09 |
| 11 | 0.10 | 4.41E-04 | 0.00 | 4.47E-02 | 0.07 | 6.97E-06 | 0.14 | 5.36E-09 | 0.11 | 1.31E-06 | 0.20 | 2.27E-08 |
| 12 | 0.00 | 1.14E-01 | 1.50 | 3.45E-01 | 0.00 | 4.31E-02 | 5.70 | 0.00E+00 | 0.00 | 1.74E-02 | 5.72 | 0.00E+00 |
| 13 | 0.23 | 4.99E-03 | 0.72 | 2.43E-01 | 0.08 | 2.69E-05 | 0.75 | 3.55E-02 | 0.06 | 7.94E-07 | 0.76 | 3.58E-02 |
| 14 | 1.59 | 3.69E-02 | 3.40 | 1.96E-03 | 0.09 | 1.61E-04 | 4.05 | 0.00E+00 | 0.36 | 3.23E-03 | 4.01 | 0.00E+00 |
| 15 | 1.73 | 4.15E-02 | 0.62 | 3.22E-01 | 4.80 | 3.79E-15 | 0.60 | 3.07E-02 | 4.89 | 0.00E+00 | 0.43 | 4.41E-03 |
| 16 | 5.67 | 9.81E-14 | 0.65 | 3.32E-01 | 3.09 | 2.12E-06 | 0.42 | 6.49E-03 | 3.83 | 5.57E-12 | 0.78 | 7.71E-02 |
| 17 | 3.06 | 2.63E-04 | 0.75 | 3.54E-01 | 2.12 | 5.32E-03 | 0.98 | 1.19E-01 | 2.29 | 5.81E-04 | 0.53 | 2.29E-02 |
| 18 | 0.00 | 1.69E-02 | 0.00 | 2.83E-01 | 0.17 | 1.63E-02 | 0.00 | 6.15E-06 | 0.13 | 3.84E-03 | 0.08 | 3.03E-05 |
| 19 | 8.04 | 0.00E+00 | 0.76 | 3.55E-01 | 1.97 | 1.14E-02 | 4.03 | 0.00E+00 | 1.79 | 1.34E-02 | 3.20 | 7.68E-12 |
| 20 | 1.24 | 1.82E-01 | 0.00 | 3.63E-01 | 0.21 | 4.18E-02 | 0.43 | 2.60E-02 | 0.17 | 1.37E-02 | 0.40 | 1.61E-02 |
| 21 | 2.23 | 2.47E-02 | 2.05 | 1.81E-01 | 2.43 | 3.79E-03 | 1.55 | 3.09E-02 | 1.73 | 3.45E-02 | 1.54 | 2.86E-02 |
| 22 | 2.77 | 3.87E-03 | 1.97 | 1.88E-01 | 1.49 | 9.17E-02 | 2.35 | 9.52E-05 | 1.83 | 2.14E-02 | 2.57 | 4.84E-06 |
| 23 | 0.00 | 5.81E-02 | 0.00 | 4.15E-01 | 0.00 | 1.62E-02 | 0.12 | 2.02E-03 | 0.00 | 4.93E-03 | 0.11 | 1.08E-03 |
| 24 | 4.75 | 1.25E-06 | 3.27 | 5.09E-02 | 1.88 | 3.64E-02 | 2.01 | 2.65E-03 | 2.02 | 1.22E-02 | 1.86 | 5.31E-03 |
| 25 | 1.36 | 2.53E-01 | 4.36 | 6.62E-02 | 0.00 | 1.15E-01 | 1.65 | 6.95E-02 | 1.47 | 1.54E-01 | 1.53 | 8.65E-02 |
| 26 | 0.00 | 1.39E-01 | 0.00 | 5.43E-01 | 0.00 | 5.71E-02 | 0.00 | 3.00E-03 | 0.00 | 2.50E-02 | 0.00 | 1.84E-03 |
| 27 | 1.14 | 2.70E-01 | 3.65 | 8.66E-02 | 0.00 | 7.55E-02 | 5.54 | 1.42E-14 | 0.31 | 1.22E-01 | 4.76 | 5.34E-12 |
| 28 | 0.00 | 3.47E-01 | 0.00 | 7.21E-01 | 1.33 | 2.57E-01 | 0.00 | 4.44E-02 | 0.52 | 2.80E-01 | 0.00 | 3.41E-02 |
| 29 | 1.28 | 3.64E-01 | 0.00 | 7.82E-01 | 0.88 | 3.71E-01 | 0.00 | 9.68E-02 | 1.38 | 2.53E-01 | 0.00 | 7.95E-02 |
| 30 | 0.00 | 4.63E-01 | 0.00 | 7.88E-01 | 0.00 | 3.28E-01 | 0.92 | 2.77E-01 | 0.00 | 2.37E-01 | 1.27 | 2.16E-01 |
| 31 | 0.00 | 6.03E-01 | 0.00 | 8.56E-01 | 1.39 | 3.58E-01 | 1.40 | 2.55E-01 | 1.08 | 3.76E-01 | 1.29 | 2.67E-01 |
| 32 | 7.89 | 5.47E-03 | 0.00 | 8.88E-01 | 9.12 | 1.39E-04 | 0.00 | 3.23E-01 | 5.69 | 3.93E-03 | 0.85 | 3.74E-01 |
| 33 | 0.00 | 8.87E-01 | 0.00 | 9.64E-01 | 0.00 | 8.40E-01 | 0.00 | 7.02E-01 | 0.00 | 7.99E-01 | 0.00 | 6.82E-01 |
| 34 | 0.00 | 7.86E-01 | 0.00 | 9.28E-01 | 0.00 | 7.06E-01 | 0.00 | 4.93E-01 | 0.00 | 6.38E-01 | 0.00 | 4.64E-01 |
| 35 | 19.14 | 2.96E-06 | 0.00 | 9.22E-01 | 15.93 | 6.14E-07 | 0.00 | 4.59E-01 | 12.42 | 2.60E-06 | 0.00 | 4.30E-01 |
| 36 | 0.00 | 8.25E-01 | 0.00 | 9.42E-01 | 0.00 | 7.57E-01 | 5.49 | 1.25E-02 | 0.00 | 6.98E-01 | 6.78 | 1.52E-03 |
| 37 | 0.00 | 9.76E-01 | 0.00 | 9.93E-01 | 0.00 | 9.66E-01 | 0.00 | 9.32E-01 | 0.00 | 9.56E-01 | 0.00 | 9.26E-01 |
| 38 | 0.00 | 8.05E-01 | 0.00 | 9.35E-01 | 3.24 | 2.33E-01 | 0.00 | 5.29E-01 | 2.53 | 2.76E-01 | 0.00 | 5.02E-01 |
| 39 | 0.00 | 8.45E-01 | 0.00 | 9.49E-01 | 0.00 | 7.83E-01 | 0.00 | 6.10E-01 | 0.00 | 7.30E-01 | 0.00 | 5.85E-01 |
| 40 | 0.00 | 8.25E-01 | 0.00 | 9.42E-01 | 0.00 | 7.57E-01 | 0.00 | 5.68E-01 | 0.00 | 6.98E-01 | 0.00 | 5.41E-01 |
| 41 | 0.00 | 8.45E-01 | 0.00 | 9.49E-01 | 0.00 | 7.83E-01 | 0.00 | 6.10E-01 | 0.00 | 7.30E-01 | 0.00 | 5.85E-01 |
| 42 | 0.00 | 8.87E-01 | 0.00 | 9.64E-01 | 0.00 | 8.40E-01 | 0.00 | 7.02E-01 | 0.00 | 7.99E-01 | 0.00 | 6.82E-01 |
| 43 | 0.00 | 9.30E-01 | 0.00 | 9.78E-01 | 0.00 | 9.01E-01 | 0.00 | 8.09E-01 | 0.00 | 8.74E-01 | 0.00 | 7.95E-01 |

**Table continues on page 161**

**Continuation of Supplementary Table 9**

| mount | $Q_{longevity}$ | | | | $Q_{all}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | up | | down | | up | | down | |
| | RF | p | RF | p | RF | p | RF | p |
| 0 | 0.10 | 0.00E+00 | 0.21 | 0.00E+00 | 0.15 | 8.58E-09 | 0.10 | 2.84E-04 |
| 1 | 0.30 | 1.56E-08 | 2.19 | 0.00E+00 | 0.18 | 2.42E-05 | 2.12 | 4.86E-03 |
| 2 | 0.37 | 2.47E-06 | 0.22 | 0.00E+00 | 0.00 | 2.29E-07 | 0.19 | 2.21E-02 |
| 3 | 0.19 | 1.09E-09 | 0.25 | 0.00E+00 | 0.07 | 7.22E-06 | 0.20 | 2.75E-02 |
| 4 | 4.87 | 0.00E+00 | 0.04 | 0.00E+00 | 7.31 | 0.00E+00 | 0.00 | 7.94E-03 |
| 5 | 0.13 | 6.92E-08 | 1.11 | 4.30E-02 | 0.00 | 8.43E-05 | 1.20 | 1.88E-01 |
| 6 | 1.40 | 9.96E-03 | 0.73 | 7.18E-03 | 1.24 | 9.01E-02 | 1.70 | 7.91E-02 |
| 7 | 0.45 | 1.40E-03 | 0.26 | 8.96E-10 | 0.12 | 1.27E-03 | 0.00 | 3.96E-02 |
| 8 | 3.62 | 0.00E+00 | 1.06 | 5.44E-02 | 2.84 | 2.01E-06 | 1.55 | 1.16E-01 |
| 9 | 0.50 | 2.40E-03 | 0.57 | 2.59E-04 | 0.54 | 5.06E-02 | 0.29 | 1.10E-01 |
| 10 | 0.00 | 3.95E-08 | 0.30 | 5.65E-07 | 0.00 | 1.41E-03 | 1.28 | 2.10E-01 |
| 11 | 0.00 | 1.90E-06 | 0.15 | 3.92E-08 | 0.00 | 6.27E-03 | 0.00 | 1.58E-01 |
| 12 | 0.00 | 5.84E-02 | 6.17 | 0.00E+00 | 0.00 | 3.35E-01 | 2.53 | 2.68E-01 |
| 13 | 0.09 | 8.61E-05 | 0.81 | 5.70E-02 | 0.22 | 4.83E-02 | 1.22 | 2.64E-01 |
| 14 | 0.10 | 4.27E-04 | 4.19 | 0.00E+00 | 0.26 | 8.09E-02 | 5.00 | 4.28E-04 |
| 15 | 4.86 | 4.18E-14 | 0.43 | 8.30E-03 | 1.52 | 1.46E-01 | 0.00 | 3.79E-01 |
| 16 | 3.41 | 4.28E-07 | 0.31 | 1.97E-03 | 5.19 | 1.29E-06 | 0.00 | 3.98E-01 |
| 17 | 2.34 | 2.52E-03 | 0.62 | 5.58E-02 | 3.25 | 4.59E-03 | 0.00 | 4.53E-01 |
| 18 | 0.19 | 2.60E-02 | 0.00 | 1.58E-05 | 0.00 | 1.28E-01 | 0.00 | 4.73E-01 |
| 19 | 1.99 | 1.35E-02 | 3.39 | 1.77E-11 | 1.86 | 1.04E-01 | 0.00 | 4.55E-01 |
| 20 | 0.24 | 5.96E-02 | 0.35 | 1.76E-02 | 0.61 | 3.19E-01 | 0.00 | 5.48E-01 |
| 21 | 2.44 | 5.37E-03 | 1.44 | 5.55E-02 | 1.26 | 2.61E-01 | 1.72 | 3.27E-01 |
| 22 | 1.65 | 7.05E-02 | 2.32 | 2.39E-04 | 1.21 | 2.64E-01 | 3.32 | 9.95E-02 |
| 23 | 0.00 | 2.41E-02 | 0.13 | 3.59E-03 | 0.00 | 2.38E-01 | 0.00 | 5.93E-01 |
| 24 | 1.56 | 9.65E-02 | 1.41 | 6.74E-02 | 0.67 | 3.37E-01 | 1.83 | 3.19E-01 |
| 25 | 0.00 | 1.42E-01 | 1.53 | 1.01E-01 | 0.00 | 4.71E-01 | 3.67 | 2.09E-01 |
| 26 | 0.00 | 7.52E-02 | 0.00 | 4.74E-03 | 0.00 | 3.69E-01 | 0.00 | 6.96E-01 |
| 27 | 0.00 | 9.68E-02 | 5.57 | 1.49E-13 | 0.00 | 4.07E-01 | 6.14 | 3.79E-02 |
| 28 | 0.00 | 2.50E-01 | 0.00 | 5.68E-02 | 0.00 | 5.86E-01 | 0.00 | 8.23E-01 |
| 29 | 0.98 | 3.74E-01 | 0.00 | 1.16E-01 | 2.51 | 2.70E-01 | 0.00 | 8.64E-01 |
| 30 | 0.00 | 3.65E-01 | 0.99 | 2.80E-01 | 0.00 | 6.78E-01 | 0.00 | 8.68E-01 |
| 31 | 1.54 | 3.47E-01 | 0.76 | 3.60E-01 | 0.00 | 7.75E-01 | 0.00 | 9.12E-01 |
| 32 | 8.06 | 1.14E-03 | 0.00 | 3.53E-01 | 15.56 | 8.28E-04 | 0.00 | 9.32E-01 |
| 33 | 0.00 | 8.54E-01 | 0.00 | 7.22E-01 | 0.00 | 9.41E-01 | 0.00 | 9.78E-01 |
| 34 | 0.00 | 7.30E-01 | 0.00 | 5.21E-01 | 0.00 | 8.86E-01 | 0.00 | 9.57E-01 |
| 35 | 17.60 | 3.43E-07 | 0.00 | 4.88E-01 | 30.18 | 6.24E-06 | 0.00 | 9.53E-01 |
| 36 | 0.00 | 7.77E-01 | 5.95 | 1.01E-02 | 0.00 | 9.08E-01 | 0.00 | 9.65E-01 |
| 37 | 0.00 | 9.69E-01 | 0.00 | 9.37E-01 | 0.00 | 9.88E-01 | 0.00 | 9.96E-01 |
| 38 | 3.58 | 2.17E-01 | 0.00 | 5.56E-01 | 0.00 | 8.97E-01 | 0.00 | 9.61E-01 |
| 39 | 0.00 | 8.02E-01 | 0.00 | 6.34E-01 | 0.00 | 9.19E-01 | 0.00 | 9.70E-01 |
| 40 | 0.00 | 7.77E-01 | 0.00 | 5.94E-01 | 0.00 | 9.08E-01 | 0.00 | 9.65E-01 |
| 41 | 0.00 | 8.02E-01 | 0.00 | 6.34E-01 | 0.00 | 9.19E-01 | 0.00 | 9.70E-01 |
| 42 | 0.00 | 8.54E-01 | 0.00 | 7.22E-01 | 0.00 | 9.41E-01 | 0.00 | 9.78E-01 |
| 43 | 0.00 | 9.10E-01 | 0.00 | 8.23E-01 | 0.00 | 9.64E-01 | 0.00 | 9.87E-01 |

**Supplementary Table 10 Gene expression mountains.** Evaluation of selected datasets, investigating the global transcriptional output of aging- and immune-relevant nematodes. Red numbers indicate significant ($p < 0.05$) overrepresentation (RF > 1). Refers to Table 19.

| mount | *daf-12(rh273)* (Fisher and Lithgow, 2006) | | | | *daf-2* (metaanalysis Evans et al., 2008) | | | | TGFbeta adults (Shaw et al., 2007) | | | | *P. aeruginosa* infection (metaanalysis Evans et al., 2008) | | | | dauers (Wang and Kim, 2001) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | up | | down | | up | | down | | up | | down | | up | | down | | | |
| | RF | p | RF | p | RF | p | RF | p | RF | p | RF | p | RF | p | RF | p | RF | p |
| 0 | 0.14 | 5.08E-03 | 0.80 | 9.54E-02 | 0.54 | 1.37E-10 | 0.48 | 1.55E-10 | 0.54 | 0.00E+00 | 0.35 | 0.00E+00 | 0.32 | 1.80E-09 | 0.47 | 3.36E-04 | 0.26 | 0.00E+00 |
| 1 | 0.47 | 1.23E-01 | 0.90 | 1.48E-01 | 1.62 | 7.46E-09 | 0.98 | 5.30E-02 | 1.72 | 0.00E+00 | 0.29 | 0.00E+00 | 1.00 | 7.49E-02 | 0.49 | 5.75E-03 | 1.71 | 4.72E-15 |
| 2 | 0.00 | 2.11E-02 | 0.15 | 6.57E-03 | 0.57 | 1.07E-05 | 0.71 | 3.45E-03 | 0.18 | 0.00E+00 | 3.64 | 0.00E+00 | 0.26 | 1.69E-06 | 0.19 | 4.83E-05 | 0.89 | 2.02E-02 |
| 3 | 0.56 | 1.78E-01 | 0.62 | 1.12E-01 | 0.56 | 1.54E-05 | 0.53 | 3.70E-05 | 0.46 | 1.06E-12 | 0.35 | 0.00E+00 | 0.54 | 3.08E-03 | 0.20 | 9.25E-05 | 0.38 | 1.10E-13 |
| 4 | 14.36 | 0.00E+00 | 0.00 | 2.26E-03 | 0.32 | 8.43E-11 | 0.56 | 1.79E-04 | 0.66 | 1.36E-05 | 0.16 | 0.00E+00 | 0.21 | 1.65E-06 | 0.78 | 8.41E-02 | 0.09 | 0.00E+00 |
| 5 | 0.00 | 9.32E-02 | 0.24 | 5.94E-02 | 0.50 | 6.34E-05 | 1.25 | 2.12E-02 | 0.44 | 2.45E-09 | 2.47 | 0.00E+00 | 0.30 | 4.00E-04 | 0.40 | 1.76E-02 | 0.90 | 3.95E-02 |
| 6 | 0.41 | 2.07E-01 | 0.90 | 1.94E-01 | 2.63 | 0.00E+00 | 0.99 | 6.91E-02 | 2.40 | 0.00E+00 | 0.18 | 0.00E+00 | 1.56 | 5.88E-03 | 0.19 | 1.19E-03 | 2.35 | 0.00E+00 |
| 7 | 0.91 | 2.72E-01 | 0.75 | 1.97E-01 | 0.29 | 1.81E-08 | 0.61 | 4.49E-03 | 0.10 | 0.00E+00 | 4.22 | 0.00E+00 | 0.06 | 1.14E-06 | 0.32 | 9.94E-03 | 0.25 | 4.32E-13 |
| 8 | 0.45 | 2.37E-01 | 4.18 | 5.10E-07 | 3.91 | 0.00E+00 | 2.54 | 0.00E+00 | 3.44 | 0.00E+00 | 0.19 | 0.00E+00 | 5.09 | 0.00E+00 | 4.58 | 0.00E+00 | 2.12 | 0.00E+00 |
| 9 | 0.00 | 8.92E-02 | 1.40 | 1.21E-01 | 0.96 | 6.16E-02 | 0.83 | 4.25E-02 | 0.73 | 1.44E-03 | 0.60 | 3.96E-09 | 0.64 | 3.10E-02 | 0.59 | 5.72E-02 | 0.89 | 3.44E-02 |
| 10 | 0.00 | 1.90E-01 | 0.34 | 1.50E-01 | 0.49 | 4.76E-04 | 0.37 | 1.76E-04 | 0.45 | 1.05E-06 | 0.32 | 0.00E+00 | 0.84 | 1.08E-01 | 0.57 | 8.90E-02 | 0.16 | 5.51E-13 |
| 11 | 0.00 | 2.77E-01 | 0.43 | 2.30E-01 | 0.13 | 4.46E-08 | 0.75 | 5.41E-02 | 0.00 | 0.00E+00 | 4.57 | 0.00E+00 | 0.00 | 7.98E-05 | 0.00 | 3.93E-03 | 0.12 | 1.48E-11 |
| 12 | 3.62 | 2.11E-01 | 0.00 | 6.05E-01 | 0.77 | 1.71E-01 | 0.98 | 2.00E-01 | 0.22 | 3.76E-03 | 1.55 | 5.70E-03 | 0.00 | 1.31E-01 | 0.84 | 3.65E-01 | 0.27 | 1.32E-02 |
| 13 | 0.00 | 3.15E-01 | 0.00 | 1.23E-01 | 0.93 | 8.67E-02 | 0.83 | 8.25E-02 | 0.94 | 6.60E-02 | 0.36 | 1.54E-11 | 0.48 | 4.69E-02 | 0.41 | 8.73E-02 | 0.94 | 7.21E-02 |
| 14 | 0.00 | 3.74E-01 | 2.26 | 6.90E-02 | 1.69 | 1.48E-03 | 0.90 | 1.05E-01 | 2.43 | 7.21E-14 | 0.08 | 0.00E+00 | 0.14 | 5.34E-03 | 2.63 | 2.30E-03 | 7.94 | 0.00E+00 |
| 15 | 4.46 | 2.52E-02 | 0.00 | 2.93E-01 | 7.43 | 0.00E+00 | 0.61 | 6.36E-02 | 1.69 | 4.94E-04 | 0.29 | 8.19E-09 | 6.14 | 0.00E+00 | 1.04 | 2.26E-01 | 3.29 | 0.00E+00 |
| 16 | 0.00 | 5.26E-01 | 2.60 | 8.08E-02 | 1.42 | 3.75E-02 | 0.74 | 1.06E-01 | 6.40 | 0.00E+00 | 0.05 | 0.00E+00 | 0.22 | 4.29E-02 | 7.31 | 4.19E-12 | 5.26 | 0.00E+00 |
| 17 | 0.00 | 5.76E-01 | 1.01 | 3.70E-01 | 4.06 | 2.82E-15 | 0.25 | 8.81E-03 | 2.29 | 1.95E-07 | 0.15 | 7.36E-11 | 1.50 | 1.05E-01 | 1.27 | 2.09E-01 | 0.27 | 4.81E-04 |
| 18 | 0.00 | 5.94E-01 | 1.06 | 3.70E-01 | 0.31 | 7.68E-03 | 0.39 | 3.32E-02 | 0.00 | 1.76E-08 | 4.22 | 0.00E+00 | 0.00 | 2.18E-02 | 0.00 | 1.06E-01 | 0.71 | 6.37E-02 |
| 19 | 0.00 | 5.77E-01 | 23.25 | 0.00E+00 | 1.26 | 8.03E-02 | 10.43 | 0.00E+00 | 2.98 | 9.94E-14 | 0.15 | 8.81E-11 | 12.85 | 0.00E+00 | 10.68 | 0.00E+00 | 4.86 | 0.00E+00 |
| 20 | 0.00 | 6.58E-01 | 0.00 | 4.68E-01 | 0.25 | 1.04E-02 | 2.11 | 5.31E-03 | 0.15 | 6.67E-05 | 2.92 | 0.00E+00 | 0.33 | 1.44E-01 | 0.00 | 1.64E-01 | 2.83 | 3.87E-08 |
| 21 | 0.00 | 6.66E-01 | 1.36 | 3.55E-01 | 2.62 | 4.76E-05 | 5.19 | 1.99E-14 | 2.20 | 2.26E-05 | 0.28 | 5.55E-06 | 5.78 | 5.42E-09 | 2.88 | 2.24E-02 | 4.92 | 0.00E+00 |
| 22 | 0.00 | 6.56E-01 | 3.94 | 3.38E-02 | 2.78 | 8.52E-06 | 3.87 | 8.83E-09 | 3.22 | 6.71E-13 | 0.86 | 6.77E-02 | 0.33 | 1.42E-01 | 5.00 | 7.11E-05 | 1.23 | 8.26E-02 |
| 23 | 0.00 | 6.95E-01 | 0.00 | 5.17E-01 | 0.59 | 9.67E-02 | 0.75 | 1.64E-01 | 0.59 | 4.41E-02 | 1.73 | 1.55E-04 | 0.38 | 1.88E-01 | 0.00 | 2.09E-01 | 1.73 | 9.33E-03 |
| 24 | 0.00 | 6.82E-01 | 4.35 | 2.69E-02 | 0.98 | 1.54E-01 | 8.38 | 0.00E+00 | 4.68 | 0.00E+00 | 0.13 | 2.72E-08 | 7.59 | 4.18E-13 | 7.36 | 8.01E-08 | 5.24 | 0.00E+00 |
| 25 | 0.00 | 8.26E-01 | 8.71 | 4.60E-03 | 1.68 | 8.15E-02 | 1.43 | 1.60E-01 | 1.78 | 2.33E-02 | 0.00 | 2.73E-06 | 5.79 | 5.87E-05 | 2.45 | 1.48E-01 | 1.16 | 1.57E-01 |
| 26 | 0.00 | 7.77E-01 | 0.00 | 6.33E-01 | 0.21 | 3.80E-02 | 0.27 | 8.69E-02 | 0.49 | 4.73E-02 | 0.13 | 7.63E-06 | 0.00 | 1.57E-01 | 0.93 | 3.70E-01 | 0.29 | 2.20E-02 |
| 27 | 0.00 | 7.96E-01 | 7.29 | 7.38E-03 | 0.23 | 5.53E-02 | 8.06 | 0.00E+00 | 6.63 | 0.00E+00 | 0.14 | 3.25E-05 | 4.85 | 2.00E-04 | 4.11 | 1.35E-02 | 6.34 | 0.00E+00 |
| 28 | 0.00 | 8.73E-01 | 4.09 | 1.93E-01 | 0.00 | 7.28E-02 | 1.00 | 2.77E-01 | 0.68 | 1.77E-01 | 2.19 | 3.51E-04 | 0.00 | 3.70E-01 | 1.73 | 3.28E-01 | 0.00 | 2.18E-02 |
| 29 | 0.00 | 9.04E-01 | 0.00 | 8.32E-01 | 0.52 | 2.84E-01 | 0.00 | 2.17E-01 | 1.52 | 1.24E-01 | 0.16 | 8.32E-03 | 0.00 | 4.75E-01 | 2.30 | 2.84E-01 | 3.28 | 9.03E-04 |
| 30 | 0.00 | 9.06E-01 | 0.00 | 8.37E-01 | 0.54 | 2.92E-01 | 4.14 | 2.31E-03 | 1.56 | 1.17E-01 | 1.84 | 1.60E-02 | 0.00 | 4.86E-01 | 0.00 | 6.54E-01 | 1.50 | 1.51E-01 |
| 31 | 0.00 | 9.37E-01 | 8.57 | 1.04E-01 | 0.82 | 3.69E-01 | 7.37 | 2.32E-05 | 5.72 | 1.09E-07 | 0.00 | 1.31E-02 | 6.41 | 9.79E-03 | 0.00 | 7.57E-01 | 4.01 | 9.41E-04 |
| 32 | 0.00 | 9.52E-01 | 0.00 | 9.15E-01 | 0.00 | 3.86E-01 | 0.00 | 4.77E-01 | 0.00 | 1.86E-01 | 2.68 | 3.59E-03 | 5.61 | 4.35E-02 | 0.00 | 8.09E-01 | 0.00 | 2.49E-01 |
| 33 | 0.00 | 9.85E-01 | 0.00 | 9.73E-01 | 0.00 | 7.43E-01 | 0.00 | 7.93E-01 | 0.00 | 5.91E-01 | 0.00 | 3.56E-01 | 0.00 | 8.93E-01 | 0.00 | 9.36E-01 | 0.00 | 6.48E-01 |
| 34 | 0.00 | 9.70E-01 | 0.00 | 9.46E-01 | 1.73 | 3.38E-01 | 2.21 | 2.98E-01 | 0.00 | 3.49E-01 | 0.00 | 1.27E-01 | 0.00 | 7.98E-01 | 0.00 | 8.76E-01 | 1.20 | 3.81E-01 |
| 35 | 0.00 | 9.67E-01 | 0.00 | 9.41E-01 | 0.00 | 5.20E-01 | 0.00 | 6.01E-01 | 1.82 | 2.13E-01 | 0.00 | 1.03E-01 | 0.00 | 7.80E-01 | 0.00 | 8.64E-01 | 1.09 | 3.84E-01 |
| 36 | 0.00 | 9.76E-01 | 0.00 | 9.56E-01 | 4.33 | 6.53E-02 | 0.00 | 6.90E-01 | 0.00 | 4.31E-01 | 0.00 | 1.92E-01 | 5.61 | 1.52E-01 | 0.00 | 8.99E-01 | 0.00 | 4.99E-01 |
| 37 | 0.00 | 9.97E-01 | 0.00 | 9.94E-01 | 0.00 | 9.42E-01 | 0.00 | 9.55E-01 | 0.00 | 9.00E-01 | 0.00 | 8.14E-01 | 0.00 | 9.78E-01 | 0.00 | 9.87E-01 | 0.00 | 9.17E-01 |
| 38 | 0.00 | 9.73E-01 | 0.00 | 9.51E-01 | 0.00 | 5.85E-01 | 4.91 | 5.33E-02 | 8.90 | 7.91E-08 | 0.00 | 1.56E-01 | 0.00 | 8.16E-01 | 0.00 | 8.88E-01 | 0.00 | 4.58E-01 |
| 39 | 0.00 | 9.79E-01 | 0.00 | 9.62E-01 | 0.00 | 6.59E-01 | 0.00 | 7.23E-01 | 0.00 | 4.79E-01 | 4.60 | 2.38E-04 | 0.00 | 8.54E-01 | 0.00 | 9.11E-01 | 0.00 | 5.45E-01 |
| 40 | 0.00 | 9.76E-01 | 0.00 | 9.56E-01 | 0.00 | 6.21E-01 | 0.00 | 6.90E-01 | 0.00 | 4.31E-01 | 4.02 | 7.75E-04 | 0.00 | 8.35E-01 | 0.00 | 8.99E-01 | 0.00 | 4.99E-01 |
| 41 | 0.00 | 9.79E-01 | 0.00 | 9.62E-01 | 0.00 | 6.59E-01 | 0.00 | 7.23E-01 | 0.00 | 4.79E-01 | 5.36 | 7.78E-06 | 0.00 | 8.54E-01 | 0.00 | 9.11E-01 | 6.87 | 1.29E-03 |
| 42 | 0.00 | 9.85E-01 | 0.00 | 9.73E-01 | 3.46 | 2.28E-01 | 0.00 | 7.93E-01 | 2.00 | 3.28E-01 | 0.00 | 3.56E-01 | 0.00 | 8.93E-01 | 0.00 | 9.36E-01 | 2.41 | 2.94E-01 |
| 43 | 0.00 | 9.91E-01 | 0.00 | 9.83E-01 | 0.00 | 8.37E-01 | 0.00 | 8.70E-01 | 0.00 | 7.29E-01 | 3.58 | 8.48E-02 | 0.00 | 9.35E-01 | 0.00 | 9.61E-01 | 0.00 | 7.71E-01 |

**Table continues on page 163**

**Continuation of Supplementary Table 10**

| mount | N2 + Resveratrol (Viswanathan et al., 2005) | | | | *daf-16* + Resveratrol (Viswanathan et al., 2005) | | | | *pmk-1* (Troemel et al., 2006) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | up | | down | | up | | down | | up | | down | |
| | RF | p | RF | p | RF | p | RF | p | RF | p | RF | p |
| 0 | 0.94 | 1.13E-01 | 1.56 | 1.88E-03 | 0.45 | 7.36E-10 | 1.35 | 1.40E-10 | 0.18 | 6.50E-04 | 0.94 | 1.84E-01 |
| 1 | 0.94 | 1.42E-01 | 1.06 | 1.05E-01 | 0.99 | 0.00E+00 | 1.28 | 2.37E-05 | 0.59 | 9.82E-02 | 0.91 | 2.29E-01 |
| 2 | 3.08 | 8.80E-07 | 0.81 | 9.83E-02 | 0.52 | 5.26E-08 | 0.80 | 9.89E-04 | 0.00 | 2.18E-03 | 0.00 | 5.15E-02 |
| 3 | 0.56 | 8.38E-02 | 1.77 | 4.00E-03 | 0.56 | 4.93E-09 | 1.18 | 2.49E-03 | 0.71 | 1.50E-01 | 1.83 | 8.27E-02 |
| 4 | 0.31 | 2.84E-02 | 0.92 | 1.24E-01 | 3.43 | 0.00E+00 | 0.23 | 0.00E+00 | 0.00 | 4.84E-03 | 0.40 | 2.01E-01 |
| 5 | 0.22 | 4.20E-02 | 0.65 | 1.01E-01 | 0.35 | 1.24E-02 | 0.90 | 2.77E-02 | 0.00 | 2.32E-02 | 0.56 | 3.01E-01 |
| 6 | 0.20 | 3.34E-02 | 0.86 | 1.39E-01 | 0.84 | 4.10E-14 | 1.47 | 7.34E-07 | 0.77 | 2.03E-01 | 0.00 | 1.45E-01 |
| 7 | 1.82 | 4.17E-02 | 0.55 | 7.78E-02 | 0.86 | 2.72E-13 | 0.67 | 1.62E-04 | 0.29 | 1.03E-01 | 0.59 | 3.14E-01 |
| 8 | 1.34 | 1.29E-01 | 0.94 | 1.50E-01 | 0.98 | 0.00E+00 | 1.07 | 3.49E-02 | 2.54 | 6.19E-03 | 4.07 | 1.22E-03 |
| 9 | 0.42 | 9.60E-02 | 2.29 | 5.90E-04 | 0.27 | 6.85E-02 | 2.38 | 0.00E+00 | 1.07 | 1.98E-01 | 0.55 | 2.96E-01 |
| 10 | 0.92 | 2.25E-01 | 2.21 | 5.35E-03 | 0.60 | 2.48E-05 | 1.23 | 8.62E-03 | 0.77 | 2.54E-01 | 1.59 | 2.28E-01 |
| 11 | 1.18 | 2.18E-01 | 0.00 | 1.39E-02 | 0.45 | 6.51E-03 | 1.44 | 4.50E-04 | 0.00 | 1.31E-01 | 0.00 | 3.74E-01 |
| 12 | 0.00 | 5.75E-01 | 1.09 | 3.69E-01 | 0.63 | 5.05E-02 | 1.15 | 1.08E-01 | 0.00 | 6.45E-01 | 9.46 | 1.77E-02 |
| 13 | 0.00 | 9.91E-02 | 2.11 | 2.30E-02 | 0.25 | 1.49E-01 | 2.59 | 0.00E+00 | 0.55 | 2.97E-01 | 2.28 | 1.61E-01 |
| 14 | 0.51 | 2.78E-01 | 3.40 | 3.22E-04 | 4.48 | 0.00E+00 | 1.49 | 6.29E-04 | 0.00 | 2.10E-01 | 0.00 | 4.70E-01 |
| 15 | 0.00 | 2.59E-01 | 0.90 | 2.70E-01 | 1.03 | 2.20E-06 | 1.30 | 2.26E-02 | 3.77 | 1.77E-02 | 0.00 | 5.96E-01 |
| 16 | 2.36 | 9.62E-02 | 0.47 | 2.55E-01 | 5.86 | 0.00E+00 | 0.75 | 3.77E-02 | 0.00 | 3.62E-01 | 2.05 | 3.03E-01 |
| 17 | 0.00 | 3.32E-01 | 1.65 | 1.64E-01 | 2.31 | 0.00E+00 | 1.49 | 6.10E-03 | 2.30 | 1.59E-01 | 2.38 | 2.79E-01 |
| 18 | 0.97 | 3.70E-01 | 0.00 | 1.77E-01 | 1.66 | 1.82E-10 | 0.10 | 2.05E-07 | 0.00 | 4.38E-01 | 0.00 | 6.71E-01 |
| 19 | 1.84 | 2.01E-01 | 1.11 | 2.71E-01 | 0.32 | 1.63E-01 | 1.50 | 5.70E-03 | 22.01 | 0.00E+00 | 0.00 | 6.57E-01 |
| 20 | 2.40 | 1.51E-01 | 0.72 | 3.49E-01 | 0.41 | 1.11E-01 | 0.44 | 5.05E-03 | 0.00 | 5.15E-01 | 0.00 | 7.25E-01 |
| 21 | 0.00 | 4.44E-01 | 0.75 | 3.53E-01 | 0.57 | 3.46E-02 | 1.50 | 1.30E-02 | 6.25 | 3.45E-03 | 0.00 | 7.32E-01 |
| 22 | 7.16 | 1.77E-04 | 0.00 | 2.45E-01 | 7.26 | 0.00E+00 | 0.57 | 1.90E-02 | 4.52 | 2.46E-02 | 0.00 | 7.24E-01 |
| 23 | 0.00 | 4.84E-01 | 0.00 | 2.98E-01 | 1.11 | 1.77E-04 | 0.07 | 7.22E-06 | 0.00 | 5.62E-01 | 0.00 | 7.57E-01 |
| 24 | 1.32 | 3.58E-01 | 1.59 | 2.27E-01 | 0.61 | 2.91E-02 | 1.25 | 6.24E-02 | 1.66 | 3.32E-01 | 0.00 | 7.46E-01 |
| 25 | 0.00 | 6.83E-01 | 0.00 | 5.30E-01 | 0.00 | 5.30E-01 | 1.67 | 2.68E-02 | 0.00 | 7.39E-01 | 6.86 | 1.27E-01 |
| 26 | 0.00 | 6.04E-01 | 0.00 | 4.32E-01 | 0.91 | 8.28E-03 | 1.47 | 3.99E-02 | 0.00 | 6.71E-01 | 5.19 | 1.60E-01 |
| 27 | 2.21 | 2.90E-01 | 1.33 | 3.58E-01 | 1.01 | 5.91E-03 | 0.70 | 1.03E-01 | 0.00 | 6.97E-01 | 0.00 | 8.40E-01 |
| 28 | 0.00 | 7.63E-01 | 0.00 | 6.38E-01 | 1.28 | 9.07E-03 | 0.98 | 1.86E-01 | 0.00 | 8.07E-01 | 0.00 | 9.02E-01 |
| 29 | 44.57 | 2.97E-13 | 0.00 | 7.14E-01 | 9.66 | 0.00E+00 | 0.00 | 1.71E-02 | 0.00 | 8.52E-01 | 0.00 | 9.25E-01 |
| 30 | 0.00 | 8.22E-01 | 0.00 | 7.21E-01 | 0.59 | 2.37E-01 | 0.27 | 8.13E-02 | 0.00 | 8.56E-01 | 0.00 | 9.27E-01 |
| 31 | 0.00 | 8.79E-01 | 0.00 | 8.07E-01 | 1.79 | 1.78E-02 | 2.46 | 2.07E-02 | 0.00 | 9.03E-01 | 0.00 | 9.52E-01 |
| 32 | 0.00 | 9.06E-01 | 0.00 | 8.49E-01 | 0.00 | 8.49E-01 | 0.54 | 2.92E-01 | 0.00 | 9.25E-01 | 0.00 | 9.63E-01 |
| 33 | 0.00 | 9.70E-01 | 0.00 | 9.50E-01 | 0.00 | 9.50E-01 | 0.00 | 5.40E-01 | 0.00 | 9.76E-01 | 0.00 | 9.88E-01 |
| 34 | 0.00 | 9.40E-01 | 0.00 | 9.03E-01 | 0.00 | 9.03E-01 | 0.00 | 2.92E-01 | 0.00 | 9.53E-01 | 0.00 | 9.77E-01 |
| 35 | 0.00 | 9.35E-01 | 0.00 | 8.94E-01 | 8.53 | 4.49E-08 | 0.78 | 3.72E-01 | 0.00 | 9.48E-01 | 0.00 | 9.74E-01 |
| 36 | 0.00 | 9.52E-01 | 0.00 | 9.22E-01 | 0.00 | 9.22E-01 | 0.00 | 3.73E-01 | 0.00 | 9.62E-01 | 0.00 | 9.81E-01 |
| 37 | 0.00 | 9.94E-01 | 0.00 | 9.90E-01 | 0.00 | 9.90E-01 | 0.00 | 8.84E-01 | 0.00 | 9.95E-01 | 0.00 | 9.98E-01 |
| 38 | 0.00 | 9.46E-01 | 21.86 | 3.45E-03 | 2.08 | 8.43E-02 | 1.92 | 2.04E-01 | 0.00 | 9.57E-01 | 0.00 | 9.79E-01 |
| 39 | 0.00 | 9.58E-01 | 0.00 | 9.31E-01 | 0.00 | 9.31E-01 | 0.00 | 4.22E-01 | 0.00 | 9.67E-01 | 0.00 | 9.84E-01 |
| 40 | 0.00 | 9.22E-01 | 0.00 | 9.22E-01 | 0.00 | 9.22E-01 | 0.00 | 3.73E-01 | 0.00 | 9.62E-01 | 0.00 | 9.81E-01 |
| 41 | 0.00 | 9.31E-01 | 0.00 | 9.31E-01 | 0.00 | 9.31E-01 | 1.23 | 3.88E-01 | 0.00 | 9.67E-01 | 0.00 | 9.84E-01 |
| 42 | 0.00 | 9.50E-01 | 0.00 | 9.50E-01 | 0.00 | 9.50E-01 | 0.00 | 5.40E-01 | 0.00 | 9.76E-01 | 0.00 | 9.88E-01 |
| 43 | 0.00 | 9.70E-01 | 0.00 | 9.70E-01 | 0.00 | 9.70E-01 | 0.00 | 6.91E-01 | 0.00 | 9.86E-01 | 0.00 | 9.93E-01 |

**Supplementary Table 11** Comparison of datasets from Q 50, 100 and 200 µM as well as pooled data-sets from $Q_{longevity}$ and $Q_{all}$ with global gene expression studies from aging- and immune-relevant literature in *C. elegans*. Numbers in red indicate significant RFs. (RF < 1, p < 0.05). Parts are displayed in Fig. 29.

| Reference | Genotype / condition | No.transcripts on array | No. DEGs in reference study | No. transcirpts identified on present array | | Q 50 µM | Q 100 µM | Q 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **DEGs total** | 623 | 2050 | 2354 | 1885 | 380 |
| | | | | | **up** | 475 | 685 | 878 | 620 | 241 |
| | | | | | **down** | 148 | 1365 | 1476 | 1261 | 88 |
| **Studies addressing the age-dependent expression changes in wild type *C. elegans*** | | | | | | | | | | |
| **Hill et al. (2000)** | | 18791 | 4221 | 3595 | all | 257 | 806 | 885 | 748 | 167 |
| | | | | | up | 204 | 249 | 304 | 231 | 106 |
| | | | | | down | 53 | 557 | 581 | 517 | 37 |
| | | | | | $RF_{all}$ | 2.16 | 2.06 | 1.97 | 2.07 | 2.30 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Lund et al. (2001)** | sterile mutant strains (*fer-15*, *spe-9*;*fer-15*, and *spe-9*;*emb-27*); 6 adult ages, pools used, three sterile mutant strains, 3-6 biological replicates per age group | 17871 | 34 | 30 | **maturity genes (expression changes on days 3 & 4)** | | | | | |
| | | | | | all | 9 | 16 | 17 | 15 | 8 |
| | | | | | up | 9 | 15 | 16 | 14 | 7 |
| | | | | | down | 0 | 1 | 1 | 0 | 0 |
| | | | | | $RF_{all}$ | 8.61 | 4.65 | 4.30 | 4.74 | 12.54 |
| | | | | | p-value | 4.93E-07 | 2.28E-08 | 1.99E-08 | 6.24E-08 | 1.43E-07 |
| | | | 164 | 151 | **aging genes (expression changes after day 4)** | | | | | |
| | | | | | all | 7 | 26 | 29 | 25 | 6 |
| | | | | | up | 7 | 11 | 15 | 11 | 5 |
| | | | | | down | 0 | 15 | 14 | 14 | 0 |
| | | | | | $RF_{all}$ | 1.33 | 1.50 | 1.46 | 1.57 | 1.87 |
| | | | | | p-value | 1.17E-01 | 9.65E-03 | 9.44E-03 | 6.94E-03 | 6.08E-02 |
| **Studies addressing the genetic background of longevity mutants and dauers** | | | | | | | | | | |
| **Murphy et al. (2003)** | Young adult *daf-2* or *age-1* vs. control, N2, or *daf-2*; *daf-16*, 1-4 replicates per comparison. Pools used. Time course of sterile mutant strain treated with *daf-2*, or *daf-2* +*daf-16* RNAi, or control vector, one array per condition per time point. | 18455 | | 240 | **up in *daf-2*, longevity mediators class I** | | | | | |
| | | | | | all | 31 | 63 | 77 | 53 | 16 |
| | | | | | up | 26 | 42 | 55 | 36 | 10 |
| | | | | | down | 5 | 21 | 20 | 14 | 1 |
| | | | | | RFall | 3.83 | 2.36 | 2.52 | 2.16 | 3.24 |
| | | | | | p-value | 1.18E-10 | 2.74E-11 | 2.51E-15 | 2.88E-08 | 2.96E-05 |
| | | | | | $RF_{up\ up}$ | 4.21 | 4.71 | 4.82 | 4.46 | 3.19 |
| | | | | | p-value | 5.55E-10 | 0.00E+00 | 0.00E+00 | 3.60E-14 | 9.35E-04 |
| | | | | | $RF_{up\ down}$ | 2.60 | 1.18 | 1.04 | 0.85 | 0.87 |
| | | | | | p-value | 3.13E-02 | 6.72E-02 | 9.17E-02 | 8.99E-02 | 3.67E-01 |
| | | | | 229 | **down in *daf-2*, longevity suppressors class II** | | | | | |
| | | | | | all | 12 | 68 | 66 | 62 | 8 |
| | | | | | up | 10 | 8 | 12 | 8 | 3 |
| | | | | | down | 2 | 60 | 54 | 53 | 1 |
| | | | | | $RF_{all}$ | 1.55 | 2.67 | 2.26 | 2.65 | 1.70 |
| | | | | | p-value | 4.09E-02 | 8.14E-15 | 4.64E-11 | 2.64E-13 | 5.37E-02 |
| | | | | | $RF_{down\ down}$ | 1.09 | 3.54 | 2.95 | 3.39 | 0.92 |
| | | | | | p-value | 2.72E-01 | 0.00E+00 | 2.18E-13 | 0.00E+00 | 3.69E-01 |
| | | | | | $RF_{down\ up}$ | 1.70 | 0.94 | 1.10 | 1.04 | 1.00 |
| | | | | | p-value | 3.75E-02 | 1.41E-01 | 1.12E-01 | 1.42E-01 | 2.27E-01 |
| **McElwee et al. (2003)** | First day adults, *daf-2(e1370)* vs. *daf2(e1370)*; *daf-16(m27)*. Pools used, 2 biological replicates, 2 technical replicates of each. | 17871 | 35 | 34 | **putative *daf-16* targets** | | | | | |
| | | | | | all | 7 | 6 | 8 | 6 | 5 |
| | | | | | up | 7 | 6 | 8 | 6 | 5 |
| | | | | | down | 0 | 0 | 0 | 0 | 0 |
| | | | | | RFall | 5.91 | 1.54 | 1.79 | 1.67 | 6.92 |
| | | | | | p-value | 1.26E-04 | 1.01E-01 | 4.18E-02 | 8.17E-02 | 6.37E-04 |
| **McElwee et al. (2004)** | First day adults, *daf-2(e1370)* or *daf-2(m577)* vs. *daf-2; daf-16*. Pools used, 5 biological replicates per genotype | 19282 | 1108 | 1039 | **up in *daf-2*** | | | | | |
| | | | | | all | 66 | 176 | 210 | 156 | 39 |
| | | | | | up | 52 | 93 | 126 | 84 | 25 |
| | | | | | down | 14 | 83 | 84 | 72 | 9 |
| | | | | | RFall | 1.97 | 1.59 | 1.66 | 1.54 | 1.90 |
| | | | | | p-value | 6.29E-08 | 6.65E-11 | 1.47E-14 | 1.05E-08 | 4.78E-05 |
| | | | | | $RF_{up\ up}$ | 2.03 | 2.52 | 2.66 | 2.51 | 1.93 |
| | | | | | p-value | 5.43E-07 | 0.00E+00 | 0.00E+00 | 2.84E-15 | 7.30E-04 |
| | | | | | $RF_{up\ down}$ | 1.76 | 1.13 | 1.06 | 1.06 | 1.90 |
| | | | | | p-value | 1.51E-02 | 2.42E-02 | 4.05E-02 | 4.38E-02 | 2.74E-02 |
| | | | | | **down in *daf-2*** | | | | | |
| | | | 779 | 736 | all | 45 | 155 | 162 | 129 | 24 |
| | | | | | up | 34 | 42 | 44 | 30 | 8 |
| | | | | | down | 11 | 113 | 118 | 98 | 7 |

**Table continues on page 165**

**Continuation Supplementary Table 11**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $RF_{all}$ | 1.89 | 1.98 | 1.80 | 1.79 | 1.65 |
| | | | | | p-value | 1.76E-05 | 0.00E+00 | 1.09E-14 | 1.37E-11 | 5.29E-03 |
| | | | | | $RF_{down\ down}$ | 1.95 | 2.17 | 2.09 | 2.04 | 2.08 |
| | | | | | p-value | 1.53E-02 | 0.00E+00 | 4.18E-15 | 5.78E-12 | 3.19E-02 |
| | | | | | $RF_{down\ up}$ | 1.88 | 1.61 | 1.31 | 1.27 | 0.87 |
| | | | | | p-value | 1.85E-04 | 7.78E-04 | 1.23E-02 | 3.27E-02 | 1.31E-01 |
| **Wang & Kim (2003)** | N2 dauer | 17088 | | 1663 | **dauer** | | | | | |
| | | | | | all | 106 | 332 | 370 | 302 | 52 |
| | | | | | up | 86 | 99 | 123 | 89 | 23 |
| | | | | | down | 20 | 233 | 247 | 213 | 29 |
| | | | | | $RF_{all}$ | 1.75 | 1.66 | 1.62 | 1.65 | 1.41 |
| | | | | | p-value | 3.16E-09 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 2.68E-03 |
| **Fisher &Lithgow (2006)** | 4 populations each of sterile strains containing *daf-12(rh61rh411)* or *daf-12(rh273)*, assayed vs mixed stage N2 reference RNA, with dye-flip for each sample | 22490 | 225 | 194 | **up in *daf-12(rh273)*** | | | | | |
| | | | | 69 | all | 23 | 42 | 42 | 41 | 21 |
| | | | | | up | 23 | 38 | 39 | 38 | 21 |
| | | | | | down | 0 | 4 | 3 | 3 | 0 |
| | | | | | $RF_{all}$ | 12.03 | 6.68 | 5.82 | 7.09 | 18.01 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | | | $RF_{up\ up}$ | 15.78 | 18.08 | 14.48 | 19.98 | 28.40 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | | | $RF_{up\ down}$ | 0.00 | 0.96 | 0.66 | 0.78 | 0.00 |
| | | | | | p-value | 6.34E-01 | 2.01E-01 | 1.68E-01 | 2.05E-01 | 7.63E-01 |
| | | | | | **down in *daf-12(rh273)*** | | | | | |
| | | | | 125 | all | 9 | 27 | 29 | 25 | 4 |
| | | | | | up | 9 | 9 | 11 | 8 | 4 |
| | | | | | down | 0 | 18 | 18 | 17 | 0 |
| | | | | | $RF_{all}$ | 2.60 | 2.37 | 2.22 | 2.39 | 1.89 |
| | | | | | p-value | 5.54E-03 | 1.21E-05 | 1.88E-05 | 2.35E-05 | 1.01E-01 |
| | | | | | $RF_{down\ down}$ | 0.00 | 2.37 | 2.19 | 2.43 | 0.00 |
| | | | | | p-value | 4.37E-01 | 3.56E-04 | 8.33E-04 | 4.05E-04 | 6.12E-01 |
| | | | | | $RF_{down\ up}$ | 3.41 | 2.36 | 2.25 | 2.32 | 2.99 |
| | | | | | p-value | 1.04E-03 | 9.41E-03 | 6.25E-03 | 1.47E-02 | 3.45E-02 |
| | | | | | **down in multiple long lived worms** | | | | | |
| | | | | 13 | all | 0 | 5 | 5 | 5 | 0 |
| | | | | | up | 0 | 0 | 0 | 0 | 0 |
| | | | | | down | 0 | 5 | 5 | 5 | 0 |
| | | | | | $RF_{all}$ | 0.00 | 4.22 | 3.67 | 4.59 | 0.00 |
| | | | | | p-value | 6.94E-01 | 3.76E-03 | 6.66E-03 | 2.63E-03 | 8.01E-01 |
| | | | | | $RF_{down\ down}$ | 0.00 | 6.34 | 5.86 | 6.86 | 0.00 |
| | | | | | p-value | 9.18E-01 | 6.39E-04 | 9.06E-04 | 4.47E-04 | 9.50E-01 |
| | | | | | $RF_{down\ up}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | p-value | 7.58E-01 | 6.69E-01 | 5.96E-01 | 6.95E-01 | 8.69E-01 |
| **Evans et al. (2008) META-ANALYSIS** | *daf- 2* summarized (Murphy et al., 2003 & McElwee et al., 2004) | 20000 | 1241 | 1155 | **broad *daf-2* up** | | | | | |
| | | | | | all | 81 | 201 | 235 | 175 | 44 |
| | | | | | up | 65 | 110 | 146 | 98 | 29 |
| | | | | | down | 16 | 91 | 89 | 76 | 9 |
| | | | | | $RF_{all}$ | 2.25 | 1.70 | 1.73 | 1.61 | 2.01 |
| | | | | | p-value | 3.19E-12 | 7.03E-15 | 0.00E+00 | 4.51E-11 | 4.99E-06 |
| | | | | | $RF_{up\ up}$ | 2.37 | 2.78 | 2.88 | 2.74 | 2.08 |
| | | | | | p-value | 5.06E-11 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 8.65E-05 |
| | | | | | $RF_{up\ down}$ | 1.87 | 1.15 | 1.04 | 1.04 | 1.77 |
| | | | | | p-value | 6.38E-03 | 1.61E-02 | 4.13E-02 | 4.51E-02 | 3.70E-02 |
| | | | | | **broad down in *daf-2*** | | | | | |
| | | | 964 | 905 | all | 51 | 197 | 203 | 169 | 29 |
| | | | | | up | 39 | 49 | 51 | 37 | 11 |
| | | | | | down | 12 | 148 | 152 | 131 | 7 |
| | | | | | $RF_{all}$ | 1.81 | 2.12 | 1.91 | 1.98 | 1.69 |
| | | | | | p-value | 1.68E-05 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 2.07E-03 |
| | | | | | $RF_{down\ down}$ | 1.79 | 2.40 | 2.28 | 2.30 | 1.76 |
| | | | | | p-value | 1.96E-02 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 5.79E-02 |
| | | | | | $RF_{down\ up}$ | 1.81 | 1.58 | 1.28 | 1.32 | 1.01 |
| | | | | | p-value | 1.31E-04 | 4.60E-04 | 1.17E-02 | 1.68E-02 | 1.23E-01 |

**Table continues on page 166**

**Continuation Supplementary Table 11**

| Reference | Genotype / condition | No.transcripts on array | No. DEGs in reference study | No. transcirpts identified on present array | | Q 50 µM | Q 100 µM | Q 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **DEGs total** | 623 | 2050 | 2354 | 1885 | 380 |
| | | | | | **up** | 475 | 685 | 878 | 620 | 241 |
| | | | | | **down** | 148 | 1365 | 1476 | 1261 | 88 |
| Shaw et al. 2007 | **TGF-beta adults** (To identify the downstream targets of the TGF-beta pathway in adulthood, they compared the dauer-constitutive mutants *daf-7(e1372)*, *daf-7(m62)*, and *daf-1(m40)* with dauer-defective mutants *daf-3(mgDf90)*, *daf-5(e1386)*, and *daf-7(e1372);daf-3(mgDf90)* double mutants at the permissive temperature, 20 C, on the first day of adulthood. Because daf-3 and daf-5 are epistatic to daf-7 and daf-1, these comparisons should identify targets that act downstream of this linear pathway.) | 20000 | 2181 | 1997 | **up in TGF-beta adults** | | | | | |
| | | | | | all | 167 | 384 | 460 | 343 | 83 |
| | | | | | up | 139 | 141 | 206 | 133 | 16 |
| | | | | | down | 28 | 243 | 254 | 209 | 50 |
| | | | | | $RF_{all}$ | 2.68 | 1.88 | 1.96 | 1.82 | 2.19 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 2.85E-12 |
| | | | | | $RF_{up\ up}$ | 2.93 | 2.06 | 2.35 | 2.15 | 0.66 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.88E-02 |
| | | | | | $RF_{up\ down}$ | 1.89 | 1.78 | 1.72 | 1.66 | 5.69 |
| | | | | | p-value | 3.79E-04 | 0.00E+00 | 0.00E+00 | 2.16E-14 | 0.00E+00 |
| | | | 4219 | 3728 | **down in TGF-beta adults** | | | | | |
| | | | | | all | 18 | 134 | 160 | 121 | 8 |
| | | | | | up | 13 | 47 | 51 | 35 | 4 |
| | | | | | down | 5 | 87 | 109 | 86 | 3 |
| | | | | | $RF_{all}$ | 0.16 | 0.35 | 0.36 | 0.34 | 0.11 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | | | p-value | | | | | |
| | | | | | $RF_{down\ down}$ | 0.18 | 0.34 | 0.40 | 0.37 | 0.18 |
| | | | | | p-value | 1.77E-08 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.68E-05 |
| | | | | | $RF_{down\ up}$ | 0.15 | 0.37 | 0.31 | 0.30 | 0.09 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Studies addressing the polyphenolic action in *C. elegans*** | | | | | | | | | | |
| Viswanathan et al. (2005) | Young adults, *daf-16* or N2, resveratrol-treated worms vs. untreated, pools used, 4 biological replicates per strain | 19282 | 330 | 315 | **up in N2+resveratrol** | | | | | |
| | | | | 118 | all | 4 | 7 | 8 | 6 | 1 |
| | | | | | up | 4 | 4 | 5 | 4 | 0 |
| | | | | | down | 0 | 3 | 3 | 2 | 0 |
| | | | | | $RF_{all}$ | 1.05 | 0.56 | 0.56 | 0.52 | 0.43 |
| | | | | | p-value | 1.98E-01 | 3.07E-02 | 2.16E-02 | 2.83E-02 | 2.26E-01 |
| | | | | | $RF_{up\ up}$ | 1.38 | 0.95 | 0.93 | 1.05 | 0.00 |
| | | | | | p-value | 1.65E-01 | 1.98E-01 | 1.77E-01 | 1.98E-01 | 2.26E-01 |
| | | | | | $RF_{up\ down}$ | 0.00 | 0.36 | 0.33 | 0.26 | 0.00 |
| | | | | | p-value | 4.02E-01 | 2.02E-02 | 1.25E-02 | 1.14E-02 | 5.82E-01 |
| | | | | 196 | **down in N2 + resveratrol** | | | | | |
| | | | | | all | 5 | 16 | 19 | 16 | 2 |
| | | | | | up | 3 | 2 | 4 | 2 | 0 |
| | | | | | down | 2 | 14 | 15 | 14 | 1 |
| | | | | | $RF_{all}$ | 0.79 | 0.77 | 0.79 | 0.84 | 0.52 |
| | | | | | p-value | 1.52E-01 | 5.25E-02 | 5.17E-02 | 7.65E-02 | 1.56E-01 |
| | | | | | $RF_{down\ down}$ | 1.33 | 1.01 | 1.00 | 1.09 | 1.12 |
| | | | | | p-value | 2.54E-01 | 1.10E-01 | 1.07E-01 | 1.04E-01 | 3.69E-01 |
| | | | | | $RF_{down\ up}$ | 0.62 | 0.29 | 0.45 | 0.32 | 0.00 |
| | | | | | p-value | 1.50E-01 | 2.12E-02 | 3.29E-02 | 3.44E-02 | 8.39E-02 |
| | | | 3495 | 3250 | **up in *daf-16* + resveratrol** | | | | | |
| | | | | 1028 | all | 95 | 185 | 223 | 178 | 77 |
| | | | | | up | 91 | 125 | 151 | 123 | 69 |
| | | | | | down | 4 | 60 | 72 | 55 | 4 |
| | | | | | $RF_{all}$ | 2.86 | 1.69 | 1.78 | 1.77 | 3.80 |
| | | | | | p-value | 0.00E+00 | 1.01E-13 | 0.00E+00 | 5.46E-15 | 0.00E+00 |
| | | | | | $RF_{up\ up}$ | 3.59 | 3.42 | 3.23 | 3.72 | 5.37 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | | | | | $RF_{up\ down}$ | 0.51 | 0.82 | 0.91 | 0.82 | 0.85 |
| | | | | | p-value | 5.78E-02 | 1.41E-02 | 3.58E-02 | 1.49E-02 | 1.89E-01 |
| | | | | 2235 | **down in *daf-16* + resveratrol** | | | | | |
| | | | | | all | 58 | 233 | 250 | 218 | 39 |
| | | | | | up | 44 | 57 | 72 | 51 | 21 |
| | | | | | down | 14 | 176 | 178 | 166 | 10 |
| | | | | | $RF_{all}$ | 0.80 | 0.98 | 0.92 | 1.00 | 0.89 |
| | | | | | p-value | 9.82E-03 | 2.77E-02 | 8.03E-03 | 3.02E-02 | 4.81E-02 |
| | | | | | $RF_{down\ down}$ | 0.82 | 1.11 | 1.04 | 1.14 | 0.98 |

**Table continues on page 167**

**Continuation Supplementary Table 11**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | p-value | 7.87E-02 | 1.03E-02 | 2.80E-02 | 7.15E-03 | 1.33E-01 |
| | | | | | $RF_{down\ up}$ | 0.80 | 0.72 | 0.71 | 0.71 | 0.75 |
| | | | | | p-value | 1.62E-02 | 9.75E-04 | 1.75E-04 | 1.22E-03 | 3.14E-02 |
| **Studies addressing the immun response of *C. elegans*** | | | | | | | | | | |
| **Troemel et al. (2006)** | *fer-15(b26)*ts;*fem-1(hc17)*; wildtype; PA14, or gacA mutant PA14 | 20000 | 308 | 289 | **4 h after *P. aeruginosa* infection up** | | | | | |
| | | | | | all | 30 | 106 | 107 | 92 | 15 |
| | | | | | up | 18 | 38 | 42 | 33 | 4 |
| | | | | | down | 12 | 68 | 65 | 59 | 6 |
| | | | | | $RF_{all}$ | 3.33 | 3.58 | 3.15 | 3.38 | 2.73 |
| | | | | | p-value | 6.58E-09 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 3.09E-04 |
| | | | | | p-value | | | | | |
| | | | | | $RF_{up\ up}$ | 2.62 | 3.84 | 3.31 | 3.68 | 1.15 |
| | | | | | p-value | 1.37E-04 | 8.99E-13 | 6.30E-12 | 9.05E-11 | 1.91E-01 |
| | | | | | $RF_{up\ down}$ | 5.61 | 3.45 | 3.05 | 3.24 | 4.72 |
| | | | | | p-value | 1.43E-06 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.45E-03 |
| | | | | | **4h after *P. aeruginosa* infection down** | | | | | |
| | | | 119 | 112 | all | 31 | 38 | 38 | 29 | 12 |
| | | | | | up | 29 | 19 | 23 | 16 | 6 |
| | | | | | down | 2 | 19 | 15 | 13 | 1 |
| | | | | | $RF_{all}$ | 8.89 | 3.31 | 2.88 | 2.75 | 5.64 |
| | | | | | p-value | 0.00E+00 | 8.12E-12 | 4.58E-10 | 2.50E-07 | 1.28E-06 |
| | | | | | $RF_{down\ down}$ | 2.41 | 2.49 | 1.81 | 1.84 | 2.03 |
| | | | | | p-value | 1.51E-01 | 1.32E-04 | 9.59E-03 | 1.33E-02 | 3.03E-01 |
| | | | | | $RF_{down\ up}$ | 10.90 | 4.95 | 4.68 | 4.61 | 4.45 |
| | | | | | p-value | 0.00E+00 | 6.72E-09 | 4.23E-10 | 2.99E-07 | 1.96E-03 |
| | | | | | **8h after *P. aeruginosa* infection up** | | | | | |
| | | | 268 | 244 | all | 18 | 89 | 91 | 78 | 13 |
| | | | | | up | 8 | 34 | 38 | 28 | 4 |
| | | | | | down | 10 | 55 | 53 | 50 | 5 |
| | | | | | $RF_{all}$ | 2.37 | 3.56 | 3.17 | 3.39 | 2.80 |
| | | | | | p-value | 4.27E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 5.83E-04 |
| | | | | | $RF_{up\ up}$ | 1.38 | 4.07 | 3.55 | 3.70 | 1.36 |
| | | | | | p-value | 9.69E-02 | 2.77E-12 | 7.78E-12 | 2.10E-09 | 1.67E-01 |
| | | | | | $RF_{up\ down}$ | 5.54 | 3.30 | 2.94 | 3.25 | 4.66 |
| | | | | | p-value | 1.21E-05 | 0.00E+00 | 5.09E-13 | 6.64E-14 | 3.74E-03 |
| | | | | | **8h after *P. aeruginosa* infection down** | | | | | |
| | | | 231 | 216 | all | 55 | 64 | 72 | 53 | 23 |
| | | | | | up | 53 | 33 | 43 | 29 | 15 |
| | | | | | down | 2 | 31 | 29 | 24 | 1 |
| | | | | | $RF_{all}$ | 8.17 | 2.89 | 2.83 | 2.60 | 5.60 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 3.68E-11 | 2.27E-11 |
| | | | | | $RF_{down\ down}$ | 1.25 | 2.10 | 1.82 | 1.76 | 1.05 |
| | | | | | p-value | 2.61E-01 | 4.05E-05 | 6.64E-04 | 2.53E-03 | 3.70E-01 |
| | | | | | $RF_{down\ up}$ | 10.33 | 4.46 | 4.53 | 4.33 | 5.76 |
| | | | | | p-value | 0.00E+00 | 4.40E-13 | 0.00E+00 | 2.61E-11 | 5.13E-08 |
| | *daf-2* vs *daf-2;pmk-1* | 20000 | | 98 | **Induced by PMK-1** | | | | | |
| | | | | | all | 10 | 33 | 36 | 31 | 8 |
| | | | | | up | 9 | 8 | 11 | 7 | 2 |
| | | | | | down | 1 | 25 | 25 | 24 | 3 |
| | | | | | $RF_{all}$ | 3.28 | 3.29 | 3.12 | 3.36 | 4.30 |
| | | | | | p-value | 7.20E-04 | 2.30E-10 | 1.05E-10 | 5.78E-10 | 4.57E-04 |
| | | | | | $RF_{up\ up}$ | 3.87 | 2.38 | 2.56 | 2.30 | 1.69 |
| | | | | | p-value | 4.28E-04 | 1.28E-02 | 2.58E-03 | 2.15E-02 | 2.16E-01 |
| | | | | | $RF_{up\ down}$ | 1.38 | 3.74 | 3.46 | 3.88 | 6.96 |
| | | | | | p-value | 3.54E-01 | 4.91E-09 | 2.28E-08 | 5.09E-09 | 8.35E-03 |
| | | | | | **Repressed by PMK-1** | | | | | |
| | | | | 47 | all | 3 | 8 | 5 | 5 | 2 |
| | | | | | up | 3 | 4 | 2 | 2 | 1 |
| | | | | | down | 0 | 4 | 3 | 3 | 0 |
| | | | | | $RF_{all}$ | 2.05 | 1.66 | 0.90 | 1.13 | 2.24 |
| | | | | | p-value | 1.22E-01 | 5.64E-02 | 1.80E-01 | 1.79E-01 | 1.65E-01 |
| | | | | | $RF_{down\ down}$ | 0.00 | 1.25 | 0.86 | 1.01 | 0.00 |
| | | | | | p-value | 7.05E-01 | 1.85E-01 | 2.24E-01 | 2.32E-01 | 8.13E-01 |
| | | | | | $RF_{down\ up}$ | 2.69 | 2.48 | 0.97 | 1.37 | 1.77 |
| | | | | | p-value | 7.54E-02 | 5.48E-02 | 2.77E-01 | 2.52E-01 | 3.25E-01 |

**Table continues on page 168**

**Continuation Supplementary Table 11**

| Reference | Genotype / condition | No.transcripts on array | No. DEGs in reference study | No. transcirpts identified on present array | | Q 50 µM | Q 100 µM | Q 200 µM | $Q_{longevity}$ | $Q_{all}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **DEGs total** | 623 | 2050 | 2354 | 1885 | 380 |
| | | | | | **up** | 475 | 685 | 878 | 620 | 241 |
| | | | | | **down** | 148 | 1365 | 1476 | 1261 | 88 |
| Shapira et al. (2006) | *P. aeruginosa* Infection | 7308 | 195 | 181 | **up after *P. aeruginosa* infection** | | | | | |
| | | | | | all | 24 | 76 | 72 | 64 | 11 |
| | | | | | up | 16 | 14 | 19 | 14 | 2 |
| | | | | | down | 8 | 62 | 53 | 50 | 4 |
| | | | | | $RF_{all}$ | 1.56 | 1.50 | 1.23 | 1.37 | 1.17 |
| | | | | | p-value | 8.69E-03 | 1.56E-05 | 5.85E-03 | 9.90E-04 | 1.09E-01 |
| | | | | | $RF_{up\ up}$ | 1.36 | 0.83 | 0.87 | 0.91 | 0.34 |
| | | | | | p-value | 4.95E-02 | 8.18E-02 | 7.90E-02 | 1.04E-01 | 4.25E-02 |
| | | | | | $RF_{up\ down}$ | 2.18 | 1.83 | 1.45 | 1.60 | 1.84 |
| | | | | | p-value | 1.93E-02 | 2.01E-07 | 8.68E-04 | 1.42E-04 | 1.07E-01 |
| | | | 33 | 33 | **down after *P. aeruginosa* infection** | | | | | |
| | | | | | all | 7 | 17 | 12 | 11 | 1 |
| | | | | | up | 7 | 14 | 11 | 10 | 1 |
| | | | | | down | 0 | 3 | 1 | 1 | 0 |
| | | | | | $RF_{all}$ | 2.49 | 1.84 | 1.13 | 1.29 | 0.58 |
| | | | | | p-value | 1.36E-02 | 2.44E-03 | 1.26E-01 | 9.19E-02 | 3.11E-01 |
| | | | | | $RF_{down\ down}$ | 0.00 | 0.49 | 0.15 | 0.18 | 0.00 |
| | | | | | p-value | 5.08E-01 | 7.17E-02 | 4.82E-03 | 1.31E-02 | 6.70E-01 |
| | | | | | $RF_{down\ up}$ | 3.26 | 4.53 | 2.77 | 3.57 | 0.92 |
| | | | | | p-value | 3.58E-03 | 4.67E-07 | 8.49E-04 | 2.24E-04 | 3.73E-01 |
| Evans et al. (2008) META-ANALYSIS | *Pseudomonas aeruginosa* infection summarized (Shapira et al. 2006 & Troemel et al. 2006) | 20000 | 482 | 446 | **up after *P. aeruginosa* infection** | | | | | |
| | | | | | all | 46 | 154 | 154 | 130 | 22 |
| | | | | | up | 31 | 50 | 60 | 44 | 6 |
| | | | | | down | 15 | 104 | 94 | 86 | 8 |
| | | | | | $RF_{all}$ | 3.31 | 3.37 | 2.93 | 3.09 | 2.60 |
| | | | | | p-value | 8.26E-13 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 3.23E-05 |
| | | | | | $RF_{up\ up}$ | 2.93 | 3.27 | 3.06 | 3.18 | 1.12 |
| | | | | | p-value | 7.52E-08 | 1.22E-13 | 6.80E-15 | 9.75E-12 | 1.58E-01 |
| | | | | | $RF_{up\ down}$ | 4.54 | 3.42 | 2.86 | 3.06 | 4.08 |
| | | | | | p-value | 9.52E-07 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 6.26E-04 |
| | | | 281 | 263 | **down after *P. aeruginosa* infection** | | | | | |
| | | | | | all | 63 | 80 | 86 | 66 | 25 |
| | | | | | up | 59 | 46 | 55 | 40 | 16 |
| | | | | | down | 4 | 34 | 31 | 26 | 2 |
| | | | | | $RF_{all}$ | 7.69 | 2.97 | 2.78 | 2.66 | 5.00 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 5.27E-14 | 3.61E-11 |
| | | | | | $RF_{down\ down}$ | 2.06 | 1.89 | 1.60 | 1.57 | 1.73 |
| | | | | | p-value | 8.53E-02 | 1.35E-04 | 2.97E-03 | 6.84E-03 | 2.13E-01 |
| | | | | | $RF_{down\ up}$ | 9.45 | 5.11 | 4.76 | 4.91 | 5.05 |
| | | | | | p-value | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.13E-07 |

**Supplementary Table 12** GO- and InterPro- cluster analysis of significant intersections between DEGs in $Q_{longevity}$ and datasets from *daf-12(rh273)* (Fisher and Lithgow, 2006), *daf-2* (Evans et al., 2008), TGF-beta adults (Shaw et al., 2007), *P. aeruginosa* infected *C. elegans* (Evans et al., 2008) and resveratrol treated *daf-16* mutants (Viswanathan et al., 2005). Only one significant ($p < 0.05$) proxy per cluster and according genes are shown. Grey fields indicate the same direction of regulation, white fields contrary. (MF: Molecular Function; BP: Biological Process; CC: Cellular Component; IP: InterPro). Refers to Fig. 29.

| $Q_{longevity}$ | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Category | Term | | No. DEGs | p-value | Genes | Category | Term | | No. DEGs | p-value | Genes |
| | up | | | | | | down | | | | | |
| ***daf-2* (meta-analysis Evans et al., 2008)** | | | | | | | | | | | | |
| up | IP | IPR014044 | SCP-like extracellular | 4 | 4.65E-04 | *scl-22, vap-2, scl-11, scl-12, scl-5*, | | | | | | |
| | MF | GO:0016491 | oxidoreductase activity | 13 | 2.55E-06 | C46H11.2, C55A6.6, *cyp-32A1*, *cyp-34A9*, *dhs-2*, F41E6.5, *fat-3*, *ftn-1*, *sod-3*, *sod-5*, *sodh-1, tyr-3*, ZC443.1, | | | | | | |
| | BP | GO:0006629 | lipid metabolic process | 6 | 6.67E-03 | *asm-2*, *fat-3*, F56F10.1, T08B1.4, *ugt-41*, *fil-1* | | | | | | |
| | BP | GO:0008340 | determination of adult life span | 7 | 3.20E-04 | *cyp-34A9*, *ftn-1*, *hsp-12.6*, *mdt-15*, *sod-3*, *sodh-1*, *spp-1*, | | | | | | |
| | BP | GO:0008152 | metabolic process | 24 | 4.01E-03 | *asm-2*, C46H11.2, C55A6.6, *cyp-34A9*, *cyp-32A1*, *cpr-2*, *dhs-2*, F38B6.4, F41E6.5, *fat-3*, *mdt-15*, *nas-9*, F56F10.1, sod-5, sod-3, *sodh-1*, T08B1.4, *tre-5*, *tyr-3*, *sams-5*, *tag-32*, *ugt-41*, W02H5.8, ZC443.1, *fil-1* | | | | | | |
| down | MF | GO:0004497 | monooxygenase activity | 3 | 1.51E-02 | *cyp-35C1*, *cyp-35A2*, *cyp-14A5* | BP | GO:0008340 | determination of adult life span | 6 | 3,14E-02 | *dod-22*, *dod-23*, *dod-19*, *ifc-2*, *jnk-1*, *pes-2.1*, *pes-2.2* |
| | | | | | | | IP | IPR000742 | EGF-like, type 3 | 4 | 3,96E-02 | F28B4.3, *nid-1*, T25C12.3, Y51H4A.25, |
| | | | | | | | CC | GO:0045111 | intermediate filament cytoskeleton | 3 | 1,55E-03 | *ifc-2*, *ifd-2*, *ifd-1* |
| | | | | | | | MF | GO:0008194 | UDP-glycosyltransferase activity | 3 | 5,23E-02 | *gly-13*, T28F3.9, Y51H4A.25 |

**Table continues on page 170**

**Continuation Supplementary Table 12**

| | $Q_{longevity}$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Category | Term<br>No. DEGs | | No. DEGs | p-value | genes | Category | Term<br>No. DEGs | | No. DEGs | p-value | genes |
| | up | | | | | | down | | | | | |
| ***daf-12(rh273)* (Fisher and Lithgow, 2006)** | | | | | | | | | | | | |
| up | IP | IPR000535 | Major sperm protein | 7 | 2,04E-10 | F44D12.3; F44D12.5; F44D12.7; C35D10.11, *msp-77*, *msp-78*, *msp-79*, *msp-38*, *msp-10*, *msp-113*, *msp-142*, *msp-152*, *msp-19*, *msp-31*, *msp-32*, *msp-36*, *msp-40*, msp-45, *msp-49*, *msp-50*, *msp-51*, *msp-53*, *msp-55*, *msp-56*, *msp-57*, *msp-59*, *msp-63*, *msp-64*, *msp-65*, *msp-74*, *msp-76*, *msp-81*, *ssp-16*, *ssp-11*, *ssp-9*, ZK1248.17 | | | | | | |
| down | IP | IPR003366 | CUB-like region | 2 | 1,86E-02 | F55G11.2, F55G11.4, | IP | IPR016186 | C-type lectin-like | 3 | 1.27E-02 | *clec-66, clec-265, clec-4* |
| ***P. aeruginosa* infection (meta-analysis Evans et al., 2008)** | | | | | | | | | | | | |
| up | MF | GO:0004497 | monooxygenase activity | 6 | 7,12E-06 | *C46H11.2, cyp-34A9, cyp-35C1, cyp-32A1, cyp-35A2, cyp-14A5,* | MF | GO:0008236 | serine-type peptidase activity | 4 | 3.60E-03 | C26B9.5, F56F10.1, K10B2.2, Y40D12A.2, |
| | IP | IPR002213 | UDP-glucuronosyl/UDP-glucosyltransferase | 3 | 1,58E-02 | *ugt-13, ugt-5, ugt-18,* | MF | GO:0003824 | catalytic activity | 13 | 1.47E-03 | ACMSD, cyp-25A1, dhs-25, gly-8, ilys-5, ilys-2, K03H6.2, M02D8.4, PLBL2_CAEEL, *sur-5*, T23F4.3, Y16B4A.2, *pho-1*, |
| | BP | GO:0006629 | lipid metabolic process | 4 | 3,28E-02 | F42F12.3, C49D10.10, *ugt-13, ugt-18* | | | | | | |
| wn | MF | GO:0016491 | oxidoreductase activity | 7 | 3,17E-03 | *alh-1, alh-5, dhs-2*, F43E2.5, sod-3, *sodh-1*, | | | | | | |

**Table continues on page 171**

**Continuation Supplementary Table 12**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| down | IP | IPR002486 | Nematode cuticle collagen, N-terminal | 3 | 4,64E-02 | *col-142*, *col-103*, *col-98* | | | | | | |
| **TGF-beta mutant adults (Shaw et al., 2007)** | | | | | | | | | | | | |
| | MF | GO:0042302 | structural constituent of cuticle | 17 | 1,56E-15 | *bli-2*, *col-89*, *col-73*, *col-103*, *col-91*, *col-2*, *col-118*, *col-90*, *col-139*, *col-51*, *col-44*, *col-98*, *col-38*, *dpy-5*, *dpy-9*, *dpy-4*, *rol-6* | IP | IPR013032 | EGF-like region, conserved site | 9 | 1,69E-03 | C49C3.4, cdh-3, F28B4.3, K05F1.10, *nid-1*, F17H10.3, R08E3.1, T25C12.3, *epi*-1 |
| | CC | GO:0005576 | extracellular region | 8 | 3,95E-03 | Y73F4A.2, *nas-9*, *nlp-30*, *scl-22*, *vap-2*, *scl-13*, *scl-2*, F32A5.4, W09D12.1, | MF | GO:0048037 | cofactor binding | 9 | 8,68E-03 | *acbp-3*, T01B11.2 , *bre-1*, C14H10.3, F56D5.3, *fasn-1*, C37E2.1 , *sptl-2*, ZC373.1 |
| up | CC | GO:0016021 | integral to membrane | 51 | 3,54E-02 | *bli-2*, C25E10.5, C30F12.5, C45B2.1, *col-2*, *col-89*, *col-44*, *col-98*, *col-91*, *col-73*, *col-164*, *col-38*, *col-139*, *col-118*, *col-51*, *col-90*, *col-103*, *cyp-32A1*, *clx-1*, *dhs-20*, *dhs-2*, *dpy-5*, *dpy-9*, *dpy-4*, F14B8.4, "F35C5.11; F35C5.12, " F59E11.7, *fipr-24*, *grl-22*, *lgc-32*, M162.5, *nlp-27*, *nlp-30*, *pgp-14*, *pgp-1*, *ptr-8*, *pmp-5*, F56F10.1, R02F2.8, *rol-6*, *sulp-2*, *snf-9*, T04F8.8, *ugt-63*, *ugt-41*, *ugt-13*, C29G2.6, Y71F9B.1, ZC410.5, ZK593.3, *elo-5* | CC | GO:0005882 | intermediate filament | 4 | 2,35E-04 | *ifp-1*, *ifc-2*, *ifd-2*, *ifd-1*, |
| | MF | GO:0003824 | catalytic activity | 32 | 5,05E-02 | C45E5.1, C46H11.2, C55A6.6, *cyp-32A1*, Y73F4A.2, *dhs-2*, *dhs-20*, F32A5.2, F38B6.4, *gstk-2*, K01C8.1, *lips-17*, *nas-9*, *ndx-2*, *pgp-14*, *pgp-1*, *pmp-5*, C05D11.5 , F56F10.1, *sod-3*, *sodh-1*, *sul-3*, T10E9.3, *ugt-13*, *ugt-41*, *ugt-63*, W02H5.8, W09D12.1, Y51H4A.5, ZC443.1, CAT-4, *acs-1*, | CC | GO:0030054 | cell junction | 6 | 9,94E-03 | *acr-8*, *cdh-3*, *inx-5*, *inx-17*, *inx-13*, *snt-1* |

**Table continues on page 172**

**Continuation Supplementary Table 12**

| | $Q_{longevity}$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Category | Term | | No. DEGs | p-value | genes | Category | Term | | No. DEGs | p-value | genes |
| | **up** | | | | | | **down** | | | | | |
| **TGF-beta mutant adults (Shaw et al., 2007)** | | | | | | | | | | | | |
| up | MF | GO:0022804 | active transmembrane transporter activity | 5 | 3,40E-02 | *pgp-14, pgp-1, pmp-5, sulp-2, snf-9* | MF | GO:0016831 | carboxy-lyase activity | 3 | 3.21E-02 | C14H10.3 *smd-1*, W05G11.6 |
| | | | | | | | BP | GO:0005975 | carbohydrate metabolic process | 9 | 5.68E-02 | *aman-3, atgp-1, bre-1*, C05C8.7, C08H9.4, *fut-6, ldh-1, ugt-12*, W05G11.6 |
| | | | | | | | IP | IPR002035 | von Willebrand factor, type A | 4 | 2.61E-02 | *clec-60*, F28B4.3, K06G5.1, T25C12.3 |
| | | | | | | | CC | GO:0005856 | cytoskeleton | 10 | 8.20E-04 | ACT-5, *hum-4, hum-5, ifp-1, ifc-2, ifd-2, ifd-1, klc-2, klp-3, osm-10,* |
| | | | | | | | IP | IPR008758 | Peptidase S28 | 3 | 7.72E-03 | C26B9.5, *pcp-5*, F56F10.1 |
| | | | | | | | MF | GO:0042625 | ATPase activity, coupled to transmembrane movement of ions | 4 | 1,55E-02 | *catp-3,mca-2*, F55F3.3, *vha-15, vha-12* |
| down | | | | | | | | | | | | |
| **Resveratrol treated *daf-16* mutants (Viswanathan et al., 2005)** | | | | | | | | | | | | |
| up | MF | GO:000519 | structural molecule activity | 33 | 2.71E-28 | C25D7.1, C34D4.3, *col-139, col-90, col-91, col-73, col-98, col-173, col-88, col-49, col-161, col-89, col-45, col-38, col-51, col-2, col-114, col-60, col-118, dpy-4*, C35D10.11, K06A5.3, *msp-10, msp-33, msp-77, rol-8, rol-6, ssp-19, ssp-10, ssp-16, ssp-11, ZK1248.17, ZK546.3* | | | | | | | |

**Table continues on page 173**

**Continuation Supplementary Table 12**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IP | IPR002486 | Nematode cuticle collagen, N-terminal | 20 | 5.08E-23 | *col-139, col-90, col-91, col-73, col-98, col-173, col-88, col-49, col-161, col-89, col-45, col-38, col-51,col-2, col-114, col-60, col-118, dpy-4, rol-8, rol-6* | | | | | | |
| | IP | IPR000535 | Major sperm protein | 13 | 8.97E-16 | C25D7.1, C34D4.3, C35D10.11, K06A5.3, *msp-77*, F58A6.9, *msp-33, ssp-10, ssp-11, ssp-19, ssp-16*, ZK1248.17, ZK546.3 | | | | | | |
| | IP | IPR004843 | Metallophosphoesterase | 3 | 3.62E-02 | F21A3.2, F52H3.6, ZK938.1 | | | | | | |
| down | | | | | | | BP | GO:0007411 | axon guidance | 5 | 3.15E-04 | *cam-1, cdh-4, egl-46, unc-44, vab-1* |
| | | | | | | | IP | IPR006209 | EGF | 4 | 1.05E-02 | *cdh-4, fbn-1, nlr-1, 02C12.1* |
| | | | | | | | BP | GO:0040018 | positive regulation of multicellular organism growth | 9 | 1.85E-02 | *catp-3, erp-72, sma-1, syd-9, sym-5, C17E4.9, unc-44, wrt-10, wrt-1* |
| | | | | | | | CC | GO:0005886 | plasma membrane | 8 | 2.85E-02 | *acr-8, C18B12.2, cdh-4, inx-2, inx-3, syd-1, wrt-1, ZC239.14* |
| | | | | | | | IP | IPR013087 | Zinc finger, C2H2-type/integrase, DNA-binding | 4 | 8.90E-03 | *egrh-1*, M03D4.4, *sem-4, syd-9* |
| | | | | | | | IP | IPR002198 | Short-chain dehydrogenase/reductase SDR | 4 | 3.41E-02 | *dhs-29, dhs-27, dhs-20, fasn-1* |
| | | | | | | | BP | GO:0018991 | oviposition | 8 | 2.95E-02 | *catp-3, dpy-27, sem-4, sma-1, syd-9, tra-4, unc-16, valv-1* |
| | | | | | | | BP | GO:0040011 | locomotion | 23 | 1.21E-02 | B0395.2, *catp-3, cam-1, dpy-27, egl-46, eor-2,* F13C5.2, F33H2.8, *fbn-1, ceh-32, inx-3, mnp-1, noah-2, osr-1, sma-1, syd-1, syd-9, sym-5,* C17E4.9, *unc-16, unc-44, wrt-10, wrt-1* |

**Supplementary Table 13 Overlaps of selected datasets.** Red numbers indicate significant overrepresentation ($p < 0.05$; $RF < 1$). Numbers in brackets display DEGs in respective condition. Refers to Table 21.

| | | *daf-12(rh273)* | | | | | | *daf-2* | | | | | | TGF-beta adults | | | | | | dauers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (69) | | | down (125) | | | "broad up" (1155) | | | "broad down" | | | up (1997) | | | down (3728) | | | up/down (1663) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | | | | 0 | 0,00 | 6,48E-01 | 6 | 1,51 | 1,05E-01 | 2 | 0,64 | 2,16E-01 | 3 | 0,44 | 5,02E-02 | 3 | 0,23 | 4,08E-04 | 6 | 1,05 | 1,67E-01 |
| | down (125) | 0 | 0,00 | 6,48E-01 | | | | 13 | 1,80 | 1,54E-02 | 34 | 6,01 | 0,00E+00 | 31 | 2,48 | 9,34E-07 | 17 | 0,73 | 3,32E-02 | 32 | 3,08 | 4,54E-09 |
| *daf-2* | "broad up" (1155) | 6 | 1,51 | 1,05E-01 | 13 | 1,80 | 1,54E-02 | | | | 4 | 0,08 | 0,00E+00 | 324 | 2,81 | 0,00E+00 | 61 | 0,28 | 0,00E+00 | 207 | 2,16 | 0,00E+00 |
| | "broad down" (905) | 2 | 0,64 | 2,16E-01 | 34 | 6,01 | 0,00E+00 | 4 | 0,08 | 0,00E+00 | | | | 216 | 2,39 | 0,00E+00 | 138 | 0,82 | 8,65E-04 | 207 | 2,75 | 0,00E+00 |
| TGF-beta adults | up (1997) | 3 | 0,44 | 5,02E-02 | 31 | 2,48 | 9,34E-07 | 324 | 2,81 | 0,00E+00 | 216 | 2,39 | 0,00E+00 | | | | 0 | 0,00 | 0,00E+00 | 430 | 2,59 | 0,00E+00 |
| | down (3728) | 3 | 0,23 | 4,08E-04 | 17 | 0,73 | 3,32E-02 | 61 | 0,28 | 0,00E+00 | 138 | 0,82 | 8,65E-04 | 0 | 0,00 | 0,00E+00 | | | | 156 | 0,50 | 0,00E+00 |
| dauers | up/down (1663) | 6 | 1,05 | 1,67E-01 | 32 | 3,08 | 4,54E-09 | 207 | 2,16 | 0,00E+00 | 207 | 2,75 | 0,00E+00 | 430 | 2,59 | 0,00E+00 | 156 | 0,50 | 0,00E+00 | | | |
| *P. aeruginosa* infection | up (446) | 2 | 1,30 | 2,58E-01 | 30 | 10,76 | 0,00E+00 | 59 | 2,29 | 1,39E-09 | 150 | 7,43 | 0,00E+00 | 94 | 2,11 | 9,43E-13 | 18 | 0,22 | 0,00E+00 | 85 | 2,29 | 1,89E-13 |
| | down (263) | 2 | 2,20 | 1,67E-01 | 9 | 5,48 | 3,54E-05 | 63 | 4,15 | 0,00E+00 | 33 | 2,77 | 8,19E-08 | 112 | 4,26 | 0,00E+00 | 12 | 0,24 | 8,11E-12 | 72 | 3,29 | 0,00E+00 |
| *pmk-1* | up (97) | 1 | 2,99 | 2,41E-01 | 15 | 24,74 | 0,00E+00 | 9 | 1,61 | 5,43E-02 | 24 | 5,47 | 5,18E-12 | 21 | 2,17 | 3,16E-04 | 5 | 0,28 | 8,08E-05 | 17 | 2,11 | 1,55E-03 |
| | down (47) | 0 | 0,00 | 8,50E-01 | 2 | 6,81 | 3,17E-02 | 3 | 1,11 | 2,28E-01 | 2 | 0,94 | 2,76E-01 | 5 | 1,07 | 1,84E-01 | 4 | 0,46 | 3,02E-02 | 3 | 0,77 | 2,05E-01 |
| N2 + resveratrol | up (118) | 0 | 0,00 | 6,64E-01 | 0 | 0,00 | 4,76E-01 | 8 | 1,17 | 1,31E-01 | 10 | 1,87 | 2,34E-02 | 20 | 1,70 | 6,51E-03 | 36 | 1,64 | 6,32E-04 | 13 | 1,32 | 6,95E-02 |
| | down (196) | 1 | 1,48 | 3,47E-01 | 0 | 0,00 | 2,91E-01 | 12 | 1,06 | 1,16E-01 | 8 | 0,90 | 1,37E-01 | 17 | 0,87 | 8,31E-02 | 18 | 0,49 | 1,01E-04 | 24 | 1,47 | 1,48E-02 |
| *daf-16* + resveratrol | up (1028) | 31 | 8,74 | 0,00E+00 | 10 | 1,56 | 5,28E-02 | 67 | 1,13 | 3,05E-02 | 40 | 0,86 | 3,88E-02 | 216 | 2,10 | 0,00E+00 | 132 | 0,69 | 7,66E-08 | 147 | 1,72 | 1,62E-11 |
| | down (2235) | 5 | 0,65 | 9,95E-02 | 14 | 1,00 | 1,13E-01 | 172 | 1,33 | 1,32E-05 | 126 | 1,25 | 1,33E-03 | 254 | 1,14 | 2,16E-03 | 388 | 0,93 | 5,92E-03 | 265 | 1,43 | 1,99E-10 |

| | | *P. aeruginosa* infection | | | | | | *pmk-1* | | | | | | N2 + resveratrol | | | | | | *daf-16* + resveratrol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (446) | | | down (263) | | | up (97) | | | down (47) | | | up (118) | | | down (196) | | | up (1028) | | | down (2235) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | 2 | 1,30 | 2,58E-01 | 2 | 2,20 | 1,67E-01 | 1 | 2,99 | 2,41E-01 | 0 | 0,00 | 8,50E-01 | 0 | 0,00 | 6,64E-01 | 1 | 1,48 | 3,47E-01 | 31 | 8,74 | 0,00E+00 | 5 | 0,65 | 9,95E-02 |
| | down (125) | 30 | 10,76 | 0,00E+00 | 9 | 5,48 | 3,54E-05 | 15 | 24,74 | 0,00E+00 | 2 | 3,30 | 3,17E-02 | 0 | 0,00 | 4,76E-01 | 0 | 0,00 | 2,91E-01 | 10 | 1,56 | 5,28E-02 | 14 | 1,00 | 1,13E-01 |
| *daf-2* | "broad up" (1155) | 59 | 2,29 | 1,39E-09 | 63 | 4,15 | 0,00E+00 | 9 | 1,61 | 5,43E-02 | 3 | 0,54 | 2,28E-01 | 8 | 1,17 | 1,31E-01 | 12 | 1,06 | 1,16E-01 | 67 | 1,13 | 3,05E-02 | 172 | 1,33 | 1,32E-05 |
| | "broad down" (905) | 150 | 7,43 | 0,00E+00 | 33 | 2,77 | 8,19E-08 | 24 | 5,47 | 5,18E-12 | 2 | 0,46 | 2,76E-01 | 10 | 1,87 | 2,34E-02 | 8 | 0,90 | 1,37E-01 | 40 | 0,86 | 3,88E-02 | 126 | 1,25 | 1,33E-03 |
| TGF-beta adults | up (1997) | 94 | 2,11 | 9,43E-13 | 112 | 4,26 | 0,00E+00 | 21 | 2,17 | 3,16E-04 | 5 | 0,52 | 1,84E-01 | 20 | 1,70 | 6,51E-03 | 17 | 0,87 | 8,31E-02 | 216 | 2,10 | 0,00E+00 | 254 | 1,14 | 2,16E-03 |
| | down (3728) | 18 | 0,22 | 0,00E+00 | 12 | 0,24 | 8,11E-12 | 5 | 0,28 | 8,08E-05 | 4 | 0,22 | 3,02E-02 | 36 | 1,64 | 6,32E-04 | 18 | 0,49 | 1,01E-04 | 132 | 0,69 | 7,66E-08 | 388 | 0,93 | 5,92E-03 |
| *P. aeruginosa* infection | up (446) | 85 | 2,29 | 1,89E-13 | 72 | 3,29 | 0,00E+00 | 17 | 2,11 | 1,55E-03 | 3 | 0,37 | 2,05E-01 | 13 | 1,32 | 6,95E-02 | 24 | 1,47 | 1,48E-02 | 147 | 1,72 | 1,62E-11 | 265 | 1,43 | 1,99E-10 |
| | down (263) | | | | 1 | 0,17 | 1,55E-02 | 32 | 14,79 | 0,00E+00 | 0 | 0,00 | 3,46E-01 | 2 | 0,76 | 2,51E-01 | 5 | 1,14 | 1,71E-01 | 17 | 0,74 | 4,02E-02 | 65 | 1,30 | 4,66E-03 |
| dauers | up/down (1663) | 1 | 0,17 | 1,55E-02 | | | | 2 | 1,57 | 2,30E-01 | 4 | 3,14 | 2,97E-03 | 1 | 0,64 | 3,30E-01 | 4 | 1,55 | 1,41E-01 | 20 | 1,48 | 2,17E-02 | 38 | 1,29 | 1,86E-02 |
| N2 + resveratrol | up (118) | 32 | 14,79 | 0,00E+00 | 2 | 1,57 | 2,30E-01 | | | | 0 | 0,00 | 7,96E-01 | 0 | 0,00 | 5,62E-01 | 0 | 0,00 | 3,84E-01 | 0 | 0,00 | 5,91E-03 | 17 | 1,57 | 1,90E-02 |
| | down (196) | 0 | 0,00 | 3,46E-01 | 4 | 6,47 | 2,97E-03 | 0 | 0,00 | 7,96E-01 | | | | 0 | 0,00 | 7,57E-01 | 1 | 2,17 | 2,93E-01 | 1 | 0,41 | 2,13E-01 | 6 | 1,14 | 1,62E-01 |
| *daf-16* + resveratrol | up (1028) | 2 | 0,76 | 2,51E-01 | 1 | 0,64 | 3,30E-01 | 0 | 0,00 | 5,62E-01 | 0 | 0,00 | 7,57E-01 | | | | 0 | 0,00 | 3,12E-01 | 42 | 6,92 | 0,00E+00 | 3 | 0,23 | 4,38E-04 |
| | down (2235) | 5 | 1,14 | 1,71E-01 | 4 | 1,55 | 1,41E-01 | 0 | 0,00 | 3,84E-01 | 1 | 1,05 | 2,93E-01 | 0 | 0,00 | 3,12E-01 | | | | 3 | 0,30 | 6,19E-03 | 75 | 3,42 | 0,00E+00 |
| *pmk-1* | up (97) | 17 | 0,74 | 4,02E-02 | 20 | 1,48 | 2,17E-02 | 0 | 0,00 | 5,91E-03 | 1 | 0,20 | 2,13E-01 | 42 | 6,92 | 0,00E+00 | 3 | 0,30 | 6,19E-03 | | | | 13 | 0,11 | 0,00E+00 |
| | down (47) | 65 | 1,30 | 4,66E-03 | 38 | 1,29 | 1,86E-02 | 17 | 1,57 | 1,90E-02 | 6 | 0,55 | 1,62E-01 | 3 | 0,23 | 4,38E-04 | 75 | 3,42 | 0,00E+00 | 13 | 0,11 | 0,00E+00 | | | |

**Supplementary Table 14 Double overlaps of $Q_{longevity}$ up-regulated genes with two other conditions, respectively.** Red numbers indicate significant over-representation ($p < 0.05$; $RF < 1$). Note: the numbers in brackets in the head line depict the overlap of $Q_{longevity}$ and respective conditions, in the column on the left the numbers indicate the total DEGs in respective conditions. Refers to Table 22.

| Qlongevity up | | *daf-12(rh273)* | | | | | | *daf-2* | | | | | | TGF-beta adults | | | | | | dauers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (38) | | | down (8) | | | "broad up" (98) | | | "broad down" | | | up (132) | | | down (35) | | | up/down (89) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | | | | 0 | 0,00 | 9,73E-01 | 2 | 5,92 | 4,04E-02 | 1 | 7,83 | 1,13E-01 | 2 | 4,39 | 6,55E-02 | 0 | 0,00 | 8,86E-01 | 3 | 9,77 | 3,36E-03 |
| | down (125) | 0 | 0,00 | 7,88E-01 | | | | 1 | 1,63 | 3,35E-01 | 3 | 12,97 | 1,50E-03 | 2 | 2,42 | 1,50E-01 | 0 | 0,00 | 8,03E-01 | 1 | 1,80 | 3,21E-01 |
| *daf-2* | "broad up" (1155) | 2 | 0,91 | 2,76E-01 | 1 | 2,16 | 3,05E-01 | | | | 0 | 0,00 | 1,10E-01 | 39 | 5,12 | 0,00E+00 | 1 | 0,49 | 2,67E-01 | 28 | 5,45 | 4,73E-14 |
| | "broad down" (905) | 1 | 0,58 | 3,10E-01 | 3 | 8,29 | 4,11E-03 | 0 | 0,00 | 1,06E-02 | | | | 7 | 1,17 | 1,41E-01 | 5 | 3,16 | 1,53E-02 | 7 | 1,74 | 6,00E-02 |
| *TGF-beta adults* | up (1997) | 2 | 0,53 | 1,59E-01 | 2 | 2,50 | 1,49E-01 | 39 | 3,99 | 5,03E-15 | 7 | 1,89 | 4,33E-02 | | | | 0 | 0,00 | 2,51E-02 | 41 | 4,61 | 0,00E+00 |
| | down (3728) | 0 | 0,00 | 3,91E-04 | 0 | 0,00 | 1,92E-01 | 1 | 0,05 | 3,55E-08 | 5 | 0,72 | 1,33E-01 | 0 | 0,00 | 1,35E-12 | | | | 4 | 0,24 | 6,97E-05 |
| dauers | up/down (1663) | 3 | 0,95 | 2,33E-01 | 1 | 1,50 | 3,62E-01 | 28 | 3,44 | 2,99E-09 | 7 | 2,28 | 2,09E-02 | 41 | 3,74 | 3,56E-14 | 4 | 1,37 | 1,70E-01 | | | |
| *P. aeruginosa* infection | up (446) | 1 | 1,18 | 3,68E-01 | 1 | 5,61 | 1,52E-01 | 8 | 3,66 | 1,23E-03 | 12 | 14,54 | 1,40E-11 | 7 | 2,38 | 1,91E-02 | 4 | 5,12 | 6,39E-03 | 10 | 5,04 | 2,44E-05 |
| | down (263) | 0 | 0,00 | 6,04E-01 | 1 | 9,51 | 9,59E-02 | 17 | 13,19 | 1,04E-14 | 2 | 4,11 | 7,24E-02 | 21 | 12,10 | 0,00E+00 | 0 | 0,00 | 6,29E-01 | 7 | 5,98 | 1,50E-04 |
| *pmk-1* | up (97) | 1 | 5,43 | 1,54E-01 | 1 | 25,77 | 3,75E-02 | 2 | 4,21 | 7,00E-02 | 1 | 5,57 | 1,51E-01 | 2 | 3,12 | 1,08E-01 | 0 | 0,00 | 8,43E-01 | 2 | 4,63 | 6,02E-02 |
| | down (47) | 0 | 0,00 | 9,14E-01 | 1 | 53,19 | 1,85E-02 | 1 | 4,34 | 1,84E-01 | 1 | 11,50 | 8,00E-02 | 1 | 3,22 | 2,29E-01 | 0 | 0,00 | 9,21E-01 | 1 | 4,78 | 1,71E-01 |
| N2 + resveratrol | up (118) | 0 | 0,00 | 7,98E-01 | 0 | 0,00 | 9,54E-01 | 1 | 1,73 | 3,27E-01 | 1 | 4,58 | 1,77E-01 | 2 | 2,57 | 1,40E-01 | 0 | 0,00 | 8,13E-01 | 2 | 3,81 | 8,14E-02 |
| | down (196) | 0 | 0,00 | 6,88E-01 | 0 | 0,00 | 9,24E-01 | 0 | 0,00 | 3,80E-01 | 0 | 0,00 | 6,94E-01 | 0 | 0,00 | 2,71E-01 | 0 | 0,00 | 7,08E-01 | 0 | 0,00 | 4,15E-01 |
| *daf-16* + resveratrol | up (1028) | 26 | 13,31 | 0,00E+00 | 1 | 2,43 | 2,84E-01 | 13 | 2,58 | 1,03E-03 | 3 | 1,58 | 1,76E-01 | 26 | 3,83 | 2,30E-09 | 1 | 0,56 | 2,99E-01 | 13 | 2,84 | 4,32E-04 |
| | down (2235) | 1 | 0,24 | 5,28E-02 | 2 | 2,24 | 1,72E-01 | 18 | 1,64 | 1,15E-02 | 7 | 1,69 | 6,40E-02 | 20 | 1,36 | 3,69E-02 | 4 | 1,02 | 2,07E-01 | 11 | 1,11 | 1,20E-01 |

| Qlongevity up | | *P. aeruginosa* infection | | | | | | *pmk-1* | | | | | | N2 + resveratrol | | | | | | *daf-16* + resveratrol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (44) | | | down (40) | | | up (7) | | | down (2) | | | up (4) | | | down (2) | | | up (123) | | | down (51) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | 1 | 6,59 | 1,31E-01 | 0 | 0,00 | 8,71E-01 | 1 | 41,41 | 2,37E-02 | 0 | 0,00 | 9,93E-01 | 0 | 0,00 | 9,86E-01 | 0 | 0,00 | 9,93E-01 | 26 | 61,27 | 0,00E+00 | 1 | 5,68 | 1,48E-01 |
| | down (125) | 1 | 3,64 | 2,10E-01 | 1 | 4,00 | 1,96E-01 | 1 | 22,86 | 4,21E-02 | 1 | 80,00 | 1,24E-02 | 0 | 0,00 | 9,75E-01 | 0 | 0,00 | 9,88E-01 | 1 | 1,30 | 3,59E-01 | 2 | 6,27 | 3,65E-02 |
| *daf-2* | "broad up" (1155) | 8 | 3,15 | 2,55E-03 | 17 | 7,36 | 1,82E-11 | 2 | 4,95 | 5,20E-02 | 1 | 8,66 | 1,09E-01 | 1 | 4,33 | 1,93E-01 | 0 | 0,00 | 8,88E-01 | 13 | 1,83 | 1,39E-02 | 18 | 6,11 | 1,81E-10 |
| | "broad down" (905) | 12 | 6,03 | 3,35E-07 | 2 | 1,10 | 2,75E-01 | 1 | 3,16 | 2,40E-01 | 1 | 11,05 | 8,64E-02 | 1 | 5,52 | 1,58E-01 | 0 | 0,00 | 9,12E-01 | 3 | 0,54 | 1,08E-01 | 7 | 3,03 | 5,81E-03 |
| TGF-beta adults | up (1997) | 7 | 1,59 | 7,73E-02 | 21 | 5,26 | 1,60E-11 | 2 | 2,86 | 1,24E-01 | 1 | 5,01 | 1,80E-01 | 2 | 5,01 | 4,85E-02 | 0 | 0,00 | 8,10E-01 | 26 | 2,12 | 1,06E-04 | 20 | 3,93 | 2,72E-08 |
| | down (3728) | 4 | 0,49 | 4,27E-02 | 0 | 0,00 | 2,59E-04 | 0 | 0,00 | 2,36E-01 | 0 | 0,00 | 6,62E-01 | 0 | 0,00 | 4,38E-01 | 0 | 0,00 | 6,62E-01 | 1 | 0,04 | 2,49E-10 | 4 | 0,42 | 1,85E-02 |
| dauers | up/down (1663) | 10 | 2,73 | 2,03E-03 | 7 | 2,10 | 2,91E-02 | 2 | 3,44 | 9,41E-02 | 1 | 6,01 | 1,52E-01 | 2 | 6,01 | 3,49E-02 | 0 | 0,00 | 8,41E-01 | 13 | 1,27 | 7,96E-02 | 11 | 2,59 | 1,92E-03 |
| *P. aeruginosa* infection | up (446) | | | | 1 | 1,12 | 3,71E-01 | 0 | 0,00 | 8,54E-01 | 0 | 0,00 | 9,56E-01 | 1 | 11,21 | 8,34E-02 | 0 | 0,00 | 9,56E-01 | 1 | 0,36 | 1,75E-01 | 7 | 6,15 | 1,14E-04 |
| | down (263) | 1 | 1,73 | 3,28E-01 | | | | 0 | 0,00 | 9,11E-01 | 2 | 76,05 | 1,72E-04 | 0 | 0,00 | 9,48E-01 | 1 | 38,02 | 2,60E-02 | 5 | 3,09 | 1,76E-02 | 4 | 5,96 | 3,96E-03 |
| *pmk-1* | up (97) | 0 | 0,00 | 8,07E-01 | 0 | 0,00 | 8,23E-01 | | | | 0 | 0,00 | 9,90E-01 | 0 | 0,00 | 9,81E-01 | 0 | 0,00 | 9,90E-01 | 0 | 0,00 | 5,49E-01 | 2 | 8,09 | 2,35E-02 |
| | down (47) | 0 | 0,00 | 9,02E-01 | 2 | 21,28 | 3,87E-03 | 0 | 0,00 | 9,84E-01 | | | | 0 | 0,00 | 9,91E-01 | 0 | 0,00 | 9,95E-01 | 1 | 3,46 | 2,18E-01 | 0 | 0,00 | 8,87E-01 |
| N2 + resveratrol | up (118) | 1 | 3,85 | 2,02E-01 | 0 | 0,00 | 7,89E-01 | 0 | 0,00 | 9,59E-01 | 0 | 0,00 | 9,88E-01 | | | | 0 | 0,00 | 9,88E-01 | 1 | 1,38 | 3,54E-01 | 0 | 0,00 | 7,39E-01 |
| | down (196) | 0 | 0,00 | 6,48E-01 | 1 | 2,55 | 2,67E-01 | 0 | 0,00 | 9,33E-01 | 0 | 0,00 | 9,80E-01 | 0 | 0,00 | 9,61E-01 | | | | 0 | 0,00 | 2,97E-01 | 1 | 2,00 | 3,06E-01 |
| *daf-16* + resveratrol | up (1028) | 1 | 0,44 | 2,34E-01 | 5 | 2,43 | 3,72E-02 | 0 | 0,00 | 6,91E-01 | 1 | 9,73 | 9,75E-02 | 1 | 4,86 | 1,76E-01 | 0 | 0,00 | 9,00E-01 | | | | 0 | 0,00 | 6,76E-02 |
| | down (2235) | 7 | 1,42 | 1,04E-01 | 4 | 0,89 | 2,00E-01 | 2 | 2,56 | 1,45E-01 | 0 | 0,00 | 7,89E-01 | 0 | 0,00 | 6,22E-01 | 1 | 4,47 | 1,99E-01 | 0 | 0,00 | 4,46E-07 | | | |

**Supplementary Table 15 Double overlaps of $Q_{longevity}$ down-regulated genes with two other conditions, respectively.** Red numbers indicate significant overrepresentation ($p < 0.05$; $RF < 1$). Note: the numbers in brackets in the head line depict the overlap of $Q_{longevity}$ and respective conditions, in the column on the left the numbers indicate the total DEGs in respective conditions. Refers to Table 23.

| Qlongevity down | | *daf-12(rh273)* | | | | | | *daf-2* | | | | | | TGF-beta adults | | | | | | dauers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (3) | | | down (17) | | | "broad up" (76) | | | "broad down" | | | up (209) | | | down (86) | | | up/down (211) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | | | | 0 | 0,00 | 9,43E-01 | 0 | 0,00 | 7,69E-01 | 0 | 0,00 | 6,35E-01 | 0 | 0,00 | 4,84E-01 | 0 | 0,00 | 7,42E-01 | 1 | 1,37 | 3,55E-01 |
| | down (125) | 0 | 0,00 | 9,81E-01 | | | | 1 | 2,11 | 2,98E-01 | 8 | 9,77 | 1,56E-06 | 5 | 3,83 | 8,11E-03 | 0 | 0,00 | 5,83E-01 | 7 | 5,31 | 3,12E-04 |
| *daf-2* | "broad up" (1155) | 0 | 0,00 | 8,37E-01 | 1 | 1,02 | 3,79E-01 | | | | 0 | 0,00 | 4,02E-04 | 21 | 1,74 | 4,86E-03 | 1 | 0,20 | 3,14E-02 | 15 | 1,23 | 7,72E-02 |
| | "broad down" (905) | 0 | 0,00 | 8,70E-01 | 8 | 10,40 | 2,74E-07 | 0 | 0,00 | 2,94E-02 | | | | 45 | 4,76 | 0,00E+00 | 13 | 3,34 | 9,48E-05 | 39 | 4,08 | 4,04E-14 |
| *TGF-beta adults* | up (1997) | 0 | 0,00 | 7,29E-01 | 5 | 2,95 | 1,73E-02 | 21 | 2,77 | 8,27E-06 | 45 | 3,44 | 2,44E-14 | | | | 0 | 0,00 | 1,15E-04 | 78 | 3,70 | 0,00E+00 |
| | down (3728) | 0 | 0,00 | 5,39E-01 | 0 | 0,00 | 2,99E-02 | 1 | 0,07 | 2,63E-06 | 13 | 0,53 | 2,51E-03 | 0 | 0,00 | 0,00E+00 | | | | 5 | 0,13 | 2,16E-13 |
| dauers | up/down (1663) | 1 | 4,01 | 2,10E-01 | 7 | 4,95 | 2,22E-04 | 15 | 2,37 | 8,76E-04 | 39 | 3,58 | 6,62E-13 | 78 | 4,49 | 0,00E+00 | 5 | 0,70 | 1,22E-01 | | | |
| *P. aeruginosa* infection | up (446) | 0 | 0,00 | 9,35E-01 | 10 | 26,38 | 4,59E-13 | 10 | 5,90 | 6,12E-06 | 37 | 12,67 | 0,00E+00 | 28 | 6,01 | 2,36E-14 | 2 | 1,04 | 2,74E-01 | 26 | 5,53 | 1,42E-12 |
| | down (263) | 1 | 25,35 | 3,84E-02 | 1 | 4,47 | 1,81E-01 | 4 | 4,00 | 1,46E-02 | 2 | 1,16 | 2,68E-01 | 12 | 4,37 | 1,79E-05 | 0 | 0,00 | 3,20E-01 | 10 | 3,60 | 3,86E-04 |
| *pmk-1* | up (97) | 0 | 0,00 | 9,86E-01 | 6 | 72,77 | 1,31E-10 | 3 | 8,14 | 5,51E-03 | 10 | 15,74 | 7,59E-10 | 8 | 7,89 | 7,30E-06 | 2 | 4,80 | 5,70E-02 | 5 | 4,89 | 3,09E-03 |
| | down (47) | 0 | 0,00 | 9,93E-01 | 0 | 0,00 | 9,61E-01 | 0 | 0,00 | 8,36E-01 | 0 | 0,00 | 7,34E-01 | 1 | 2,04 | 3,03E-01 | 0 | 0,00 | 8,16E-01 | 0 | 0,00 | 6,07E-01 |
| *N2 + resveratrol* | up (118) | 0 | 0,00 | 9,82E-01 | 0 | 0,00 | 9,04E-01 | 0 | 0,00 | 6,37E-01 | 1 | 1,29 | 3,60E-01 | 0 | 0,00 | 2,88E-01 | 0 | 0,00 | 6,01E-01 | 0 | 0,00 | 2,85E-01 |
| | down (196) | 0 | 0,00 | 9,71E-01 | 0 | 0,00 | 8,46E-01 | 0 | 0,00 | 4,72E-01 | 1 | 0,78 | 3,58E-01 | 1 | 0,49 | 2,64E-01 | 1 | 1,19 | 3,66E-01 | 5 | 2,42 | 3,91E-02 |
| *daf-16* + resveratrol | up (1028) | 0 | 0,00 | 8,54E-01 | 1 | 1,14 | 3,76E-01 | 4 | 1,02 | 2,01E-01 | 8 | 1,19 | 1,28E-01 | 16 | 1,49 | 3,13E-02 | 1 | 0,23 | 4,96E-02 | 14 | 1,29 | 7,04E-02 |
| | down (2235) | 0 | 0,00 | 7,01E-01 | 2 | 1,05 | 2,87E-01 | 15 | 1,77 | 1,08E-02 | 15 | 1,02 | 1,09E-01 | 26 | 1,11 | 7,11E-02 | 19 | 1,98 | 1,58E-03 | 41 | 1,74 | 1,46E-04 |

| Qlongevity down | | *P. aeruginosa* infection | | | | | | *pmk-1* | | | | | | N2 + resveratrol | | | | | | *daf-16* + resveratrol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | up (86) | | | down (26) | | | up (23) | | | down (3) | | | up (2) | | | down (14) | | | up (55) | | | down (166) | | |
| | | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p | DEGs | RF | p |
| *daf-12(rh273)* | up (69) | 0 | 0,00 | 7,42E-01 | 1 | 11,15 | 8,24E-02 | 0 | 0,00 | 9,24E-01 | 0 | 0,00 | 9,90E-01 | 0 | 0,00 | 9,93E-01 | 0 | 0,00 | 9,53E-01 | 0 | 0,00 | 8,27E-01 | 0 | 0,00 | 5,62E-01 |
| | down (125) | 10 | 18,60 | 1,44E-10 | 1 | 6,15 | 1,39E-01 | 6 | 41,74 | 4,82E-09 | 0 | 0,00 | 9,81E-01 | 0 | 0,00 | 9,88E-01 | 0 | 0,00 | 9,16E-01 | 1 | 2,91 | 2,46E-01 | 2 | 1,93 | 1,92E-01 |
| *daf-2* | "broad up" (1155) | 10 | 2,01 | 1,58E-02 | 4 | 2,66 | 4,49E-02 | 3 | 2,26 | 1,04E-01 | 0 | 0,00 | 8,37E-01 | 0 | 0,00 | 8,88E-01 | 0 | 0,00 | 4,35E-01 | 4 | 1,26 | 1,83E-01 | 15 | 1,56 | 2,64E-02 |
| | "broad down" (905) | 37 | 9,51 | 0,00E+00 | 2 | 1,70 | 2,19E-01 | 10 | 9,61 | 2,16E-08 | 0 | 0,00 | 8,70E-01 | 1 | 11,05 | 8,64E-02 | 1 | 1,58 | 3,47E-01 | 8 | 3,21 | 2,40E-03 | 15 | 2,00 | 4,89E-03 |
| *TGF-beta adults* | up (1997) | 28 | 3,26 | 6,58E-09 | 12 | 4,62 | 2,13E-06 | 8 | 3,48 | 9,93E-04 | 1 | 3,34 | 2,43E-01 | 0 | 0,00 | 8,10E-01 | 1 | 0,72 | 3,56E-01 | 16 | 2,91 | 4,67E-05 | 26 | 1,57 | 6,29E-03 |
| | down (3728) | 2 | 0,12 | 3,67E-06 | 0 | 0,00 | 4,67E-03 | 2 | 0,47 | 1,15E-01 | 0 | 0,00 | 5,39E-01 | 0 | 0,00 | 6,62E-01 | 1 | 0,38 | 1,79E-01 | 1 | 0,10 | 1,47E-04 | 19 | 0,61 | 3,91E-03 |
| dauers | up/down (1663) | 26 | 3,64 | 2,92E-09 | 10 | 4,63 | 2,06E-05 | 5 | 2,61 | 2,80E-02 | 0 | 0,00 | 7,71E-01 | 0 | 0,00 | 8,41E-01 | 5 | 4,30 | 3,63E-03 | 14 | 3,06 | 9,12E-05 | 41 | 2,97 | 1,14E-10 |
| *P. aeruginosa* infection | up (446) | | | | 0 | 0,00 | 5,56E-01 | 15 | 29,25 | 0,00E+00 | 0 | 0,00 | 9,35E-01 | 0 | 0,00 | 9,56E-01 | 0 | 0,00 | 7,29E-01 | 5 | 4,08 | 6,14E-03 | 9 | 2,43 | 8,18E-03 |
| | down (263) | 0 | 0,00 | 3,20E-01 | | | | 0 | 0,00 | 7,37E-01 | 0 | 0,00 | 9,61E-01 | 0 | 0,00 | 9,74E-01 | 1 | 5,43 | 1,55E-01 | 1 | 1,38 | 3,54E-01 | 3 | 1,37 | 1,98E-01 |
| *pmk-1* | up (97) | 15 | 35,96 | 0,00E+00 | 0 | 0,00 | 8,81E-01 | | | | 0 | 0,00 | 9,86E-01 | 0 | 0,00 | 9,90E-01 | 0 | 0,00 | 9,34E-01 | 0 | 0,00 | 7,65E-01 | 6 | 7,45 | 1,42E-04 |
| | down (47) | 0 | 0,00 | 8,16E-01 | 0 | 0,00 | 9,41E-01 | 0 | 0,00 | 9,47E-01 | | | | 0 | 0,00 | 9,95E-01 | 0 | 0,00 | 9,68E-01 | 0 | 0,00 | 8,78E-01 | 1 | 2,56 | 2,66E-01 |
| *N2 + resveratrol* | up (118) | 0 | 0,00 | 6,01E-01 | 0 | 0,00 | 8,57E-01 | 0 | 0,00 | 8,73E-01 | 0 | 0,00 | 9,82E-01 | | | | 0 | 0,00 | 9,20E-01 | 0 | 0,00 | 7,22E-01 | 0 | 0,00 | 3,73E-01 |
| | down (196) | 0 | 0,00 | 4,28E-01 | 1 | 3,92 | 1,99E-01 | 0 | 0,00 | 7,97E-01 | 0 | 0,00 | 9,71E-01 | 0 | 0,00 | 9,80E-01 | | | | 0 | 0,00 | 5,81E-01 | 3 | 1,84 | 1,42E-01 |
| *daf-16* + resveratrol | up (1028) | 5 | 1,13 | 1,74E-01 | 1 | 0,75 | 3,57E-01 | 0 | 0,00 | 2,97E-01 | 0 | 0,00 | 8,54E-01 | 0 | 0,00 | 9,00E-01 | 0 | 0,00 | 4,78E-01 | | | | 1 | 0,12 | 1,37E-03 |
| | down (2235) | 9 | 0,94 | 1,36E-01 | 3 | 1,03 | 2,38E-01 | 6 | 2,33 | 2,62E-02 | 1 | 2,98 | 2,65E-01 | 0 | 0,00 | 7,89E-01 | 3 | 1,92 | 1,38E-01 | 1 | 0,16 | 1,02E-02 | | | |

**Supplementary Table 16** Studies with *C. elegans* exposed to natural substances (Saul et al., 2009).

| Substance | Observations in *C. elegans* | Supposed mechanisms | References |
|---|---|---|---|
| EGCG | • No lifespan extension<br>• Enhanced thermal & oxidative stress resistance<br>• Enhanced chemotaxis index<br>• Decreased $H_2O_2$ level in N2, but not in *daf-16* mutants<br>• Attenuated decline in pharyngeal pumping behavior<br>• Delayed movement decline & Aβ-induced muscle paralysis<br>• Up-regulation of *sod-3, skn-1* & *hsp-16.2* expression | • Increase of energy expenditure<br>• ROS scavenging activity<br>• Up-regulation of *sod-3, hsp-16.2* & *skn-1* | Brown et al., 2006; Zhang et al., 2009 |
| | • Increased lifespan of N2 & *mev-1* mutants<br>• Enhanced oxidative stress resistance<br>• Reduced intracellular $H_2O_2$ level & juglone-induced expression of *hsp-16.2* | • Antioxidant properties | Abbas & Wink, 2008 |
| Quercetin | • Enhanced resistance to oxidative & thermal stress<br>• Increased lifespan in N2 & *akt-2, daf-12, daf-16, jnk-1, mev-1, nhr-8, osr-1, sir-2.1* & *skn-1* mutants<br>• No increased lifespan in *age-1, daf-2, sek-1* & *unc-43* mutants<br>• No effects on reproduction or body length | • Antioxidant capacities<br>• UNC-43/SEK-1 pathway | Pietsch et al., 2008; Saul et al., 2008 |
| | • Increased thermal & oxidative stress resistance<br>• Reduction of SOD-3 expression & lipofuscin accumulation<br>• Reduction of generated ROS during thermal stress<br>• Reduction of *gst-4* promoter induction during juglone treatment<br>• Translocation of DAF-16 from the cytosol to the nucleus | • Antioxidant capacities<br>• Modulation of DAF-16 activity | Kampkötter et al., 2007b, 2008 |
| Resveratrol | • Increased lifespan in N2 & *daf-16* mutants<br>• No lifespan extension in *sir-2.1* mutants<br>• Stimulated deacetylation activity of SIR-2.1<br>• Induced expression of *cyp-13A6* & several *abu* & *pqn* genes<br>• No lifespan extension in *abu-11* RNAi assay | • Modulation of stress response via SIR-2.1 & ABU-11 | Wood et al., 2004; Viswanathan et al., 2005 |
| | • Increased lifespan & oxidative stress resistance<br>• Changes in reproduction pattern, but unchanged overall progeny<br>• No changes in pharyngeal pumping rate or body length | • Delay of the onset of the exponential increase in mortality | Gruber et al., 2007 |
| | • Variable lifespan extension in N2 & *sir-2.1* mutants | • SIR-2.1 independently activation of phase 2 drug detoxification<br>• AMP kinase activation | Bass et al., 2007 |
| Blueberry extract | • Increased lifespan at 20°C & 25°C, but not at 15°C<br>• Increased pharyngeal pumping rate & thermal (but not oxidative) stress resistance<br>• Delayed age related changes in *hsp* expression<br>• No lifespan extension in *mev-1, osr-1, sek-1* & *unc-43* mutants, but in *sir-2.1,, daf-16 & skn-1 mutants* | • OSR-1/UNC-43/SEK-1 pathway | Wilson et al., 2006 |
| Fisetin | • Increased thermal resistance<br>• Reduced ROS accumulation under heat stress<br>• Translocation of DAF-16 from the cytosol to the nucleus | • Modulation of DAF-16 activity<br>• Antioxidative capacity | Kampkötter at al., 2007a |
| Wisconsin Ginseng | • *No significant lifespan extension*<br>• *Reduced age dependent declines of locomotory rate, pharyngeal pumping & body wall muscle cells* | | Cao et al., 2007 |
| Gingko Biloba (EGb 761) | • Increased lifespan<br>• Increased thermal & oxidative stress resistance in N2 & *mev-1* mutants<br>• Reduced ROS accumulation, *gst-4* promoter activity & catalase transcription<br>• *Reduced age dependent declines of locomotory rate, pharyngeal pumping & body wall muscle cells*<br>• Suppression of heat shock induced *hsp-16.2* expression | • Antioxidative properties<br>• Modulation of *hsp-16.2* expression | Wu et al., 2002; Strayer et al., 2003; Cao et al., 2007; Kampkötter et al., 2007c |
| Vitamin E | • Increased lifespan<br>• Decreased & delayed reproduction<br>• No lifespan extension in *mev-1*<br>• No modulation of mitochondrial superoxide anion production in N2 & *mev-1* | • Slow down of reproduction & development | Harrington & Harley, 1988; Ishii et al., 2004 |
| Tocopherol | • Lifespan extension with γ-tocopherol, but not with α -tocopherol | | Adachi & Ishii, 2000; Zou et al., 2007 |
| Tocotrienol | • Increased lifespan<br>• Enhanced UVB stress resistance | • Reduction of ROS mediated damage | Adachi & Ishii, 2000 |

**Table continues on page 178**

**Continuation Supplementary Table 16**

| | | | |
|---|---|---|---|
| Coenzyme Q10 | • Increased lifespan in N2 & *mev-1* mutants<br>• Modulation of mitochondrial superoxide anion production in N2 & *mev-1*<br>• Significant suppression of apoptosis in *mev-1* embryos | • Reduction of oxidative stress in mitochondria | Ishii et al., 2004 |
| Kaempferol | • Increased thermal resistance<br>• Reduced ROS accumulation under heat stress<br>• Reduced lipofuscin autofluorescence<br>• Translocation of DAF-16 from the cytosol to the nucleus | • Modulation of DAF-16 activity<br>• Antioxidative capacity | Kampkötter et al., 2007a |
| Rutin | • No thermal stress resistance<br>• Reduction of generated ROS during thermal stress<br>• Reduced *gst-4* promoter induction during juglone treatment<br>• No significant effect on DAF-16 distribution<br>• No altered lipofuscin accumulation | | Kampkötter et al., 2007b |
| Humic Substances | • Lifespan extension in low to moderate concentrations<br>• Up-regulation of stress-defense related genes | • Hormesis<br>• Modulation of stress defense system | Steinberg et al., 2007 |
| α-lipoic acid | • Increased life extension<br>• Attenuated $H_2O_2$ level in N2 & *daf-16* mutants<br>• No attenuation of the rate of decline in pharyngeal pumping behavior<br>• Enhancement of the chemotaxis index<br>• No lifespan extension in *daf-16* mutants<br>• No delay in Aβ-induced muscle paralysis | • Insulin/IGF-1 pathway | Brown et al., 2006 |

**Supplementary Information 1**

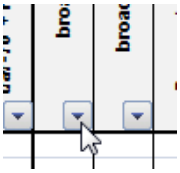
# HOW TO USE THE FILTERTABLE? - Short description

The filtertable can be found online under following web-address:

**https://docs.google.com/leaf?id=0B-ebpwy-wEV6MNGQ1MzU1ZGYtYjZhYS00ZGY2LWIwNTYtMjM5MGE1M2ZhMzJi&hl=en_US&authkey=CNuBhcgP**

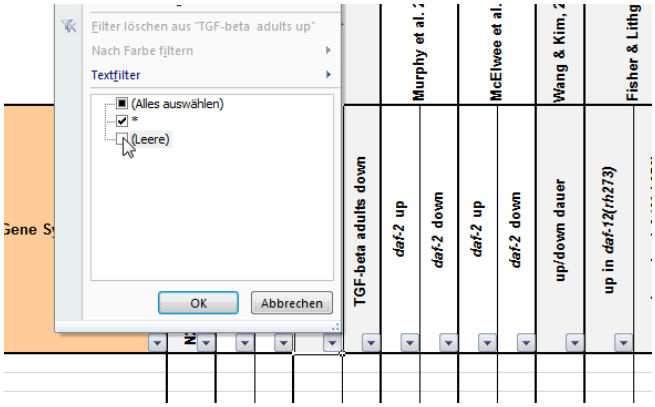
This filter table contains the full genome (22 548 transcripts) fold change expression values for Q 50, 100 and 200 µM treated wild type *C. elegans* relative to control. Statistical significance of genes is indicated by an "X" in the row "Statistical Significance", labeled with respective concentration. Additionally the filter table highlights the DEGs of 11 DNA-microarray studies formerly conducted in *C. elegans*. The genes which are regulated in respective study are marked with an "*" in according row. Furthermore there are rows for all 44 gene expression mountains (according to Kim et al., 2001) and various gene-classes and gene groups. Genes that belong to respective mountains or groups are marked with an "*".
Based on the filter function of Microsoft excel this table can help to compare the various conditions with each other, determine overlaps and exclusively regulated genes. The numbers in the line "all DEGs in respective condition" display the total number of DEGs in respective filter condition. This number does not change. The numbers in "regulated in filter conditions" varies dependent on the filter adjustments; they show how many DEGs are contained in respective group (e.g. the overlap to other studies) Selected DNA-microarray studies on which will be mainly responded in the thesis are highlighted in grey

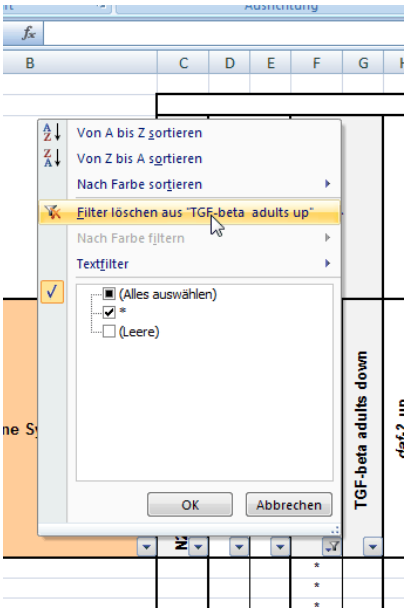
1. To filter for a certain condition klick the **filter sign** on respective condition.



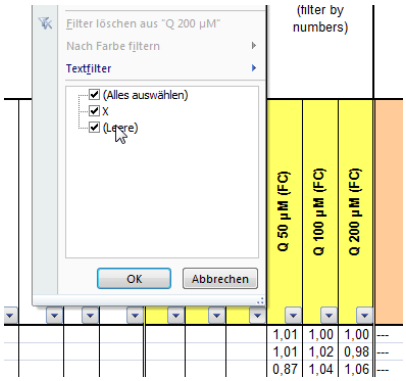
2. For DEGs in a "**microarray study**", "**gene expression mountain"** or "**gene-class"** or "**-group"**:

filter for "*****",
to exclude according DEGs filter for "**Leere**",
to delete a set filter klick "**Filter löschen**"

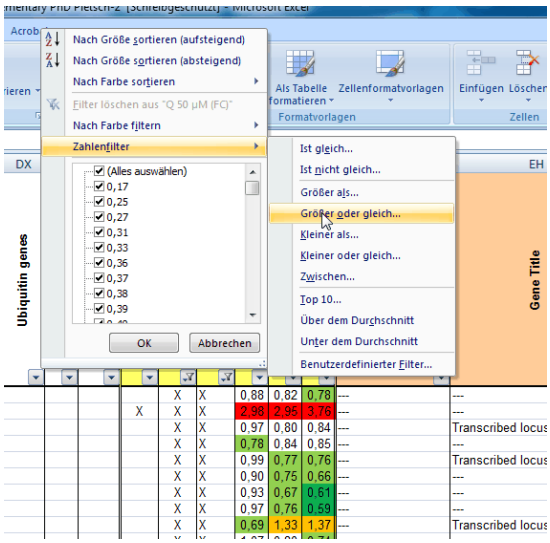


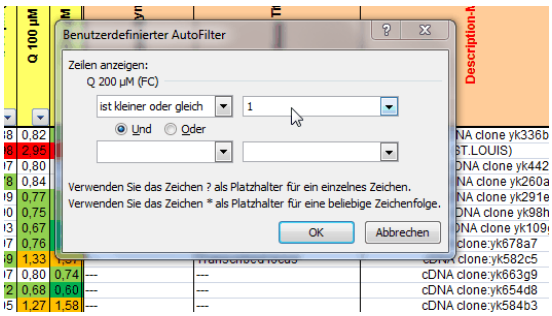



3. To show **significant regulated genes** in **Q concentrations**:
   filter for "**X**" in the row "**Statistical Significance**"

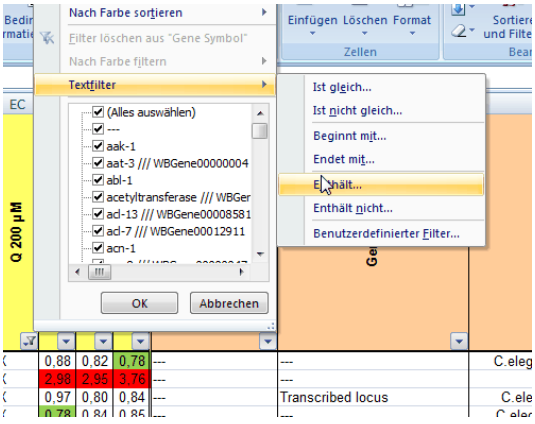



4. To filter for **Fold change values in Q concentrations:**
   use the "**Zahlenfilter**" and
   **enter numbers**: e. g. "> 1" or "< 1"




5. To search for **single genes or descriptive** words:
   use the "**Textfilter**" in "**Annotations**" row
   **enter the word** you are looking for in the filter.

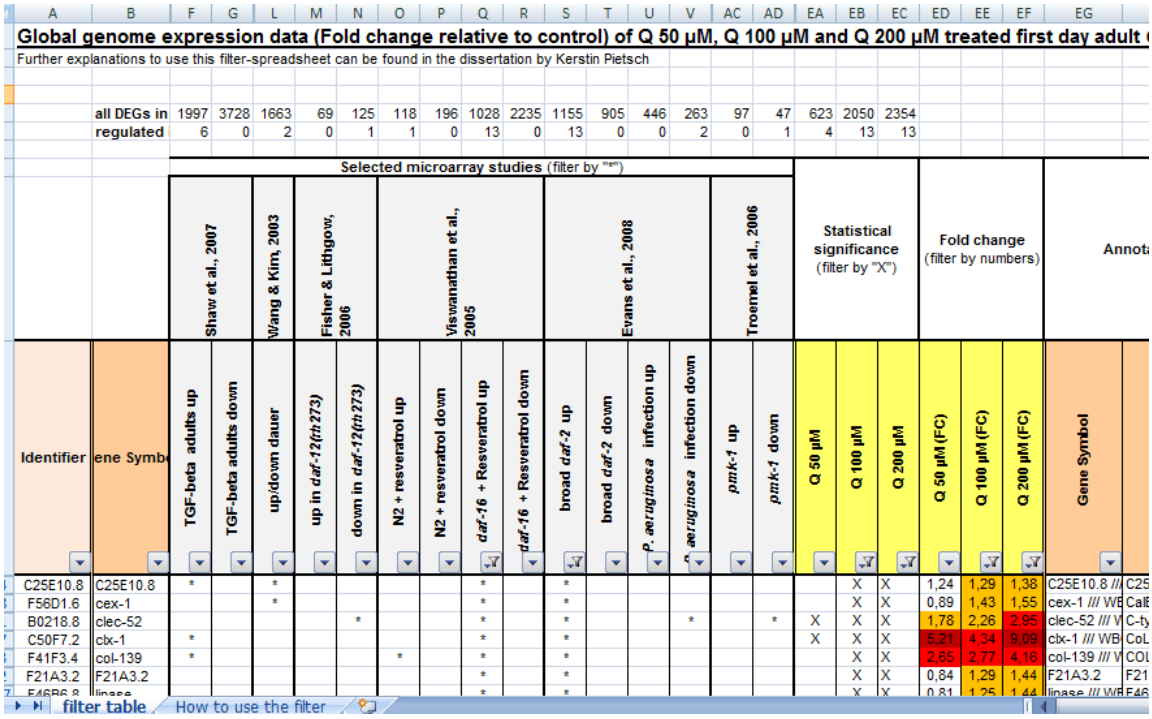


6. It is possible to **filter in diverse conditions in parallel**.

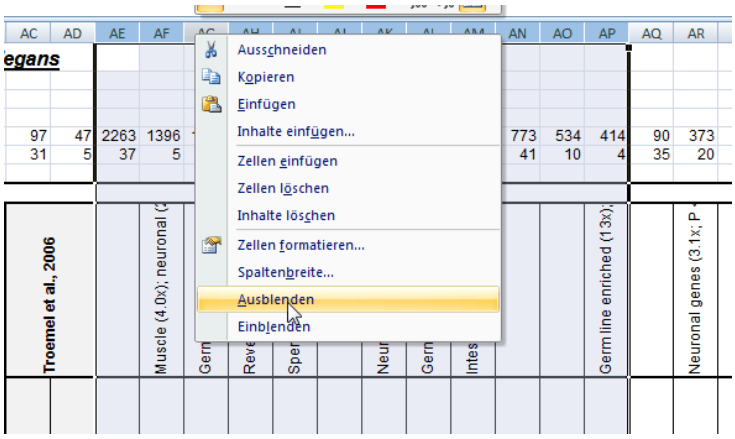
7. The **numbers above the table** indicate:

-"**all DEGs in respective condition**" displays the number of regulated DEGs in respective condition

- "**regulated in filter conditions**" **varies dependent on the filter adjustment**; it shows how many DEGs are contained in the respective group under total filter calibrations




8. For better overview, the **table view** can be **reduced** by marking the complete row which is not be needed, right klick with the mouse, klick "**AUSBLENDEN**"; later on information can be showed again with "**EINBLENDEN**"



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Vielen Dank.

# List of Publications

**Pietsch K**, Saul N, Swain S, Menzel R, Steinberg CEW, Stürzenbaum SR (**2011**) Concentration dependent global transcriptional responses to longevity mediating polyphenolic compounds Quercetin and Tannic acid in *Ceanorhabditis elegans*. (In preparation)

**Pietsch K**, Saul N, Chakrabarti S, Stürzenbaum SR, Menzel R, Steinberg CEW (**2011**) Hormetins, Antioxidants and Prooxidants: Defining Quercetin-, Caffeic acid- and Rosmarinic acid- mediated life extension in *C. elegans*. *Biogerontology*. [Epub ahead of print], DOI: 10.1007/s10522-011-9334-7

Saul N, **Pietsch K**, Menzel R, Stürzenbaum SR, Steinberg CEW (**2011**) The diversity of tannin action in *C. elegans*: Between toxicity and longevity. (under Review)

**Pietsch K**, Hofmann S, Henkel R, Saul N, Menzel R, Steinberg CEW (**2010**) The plant polyphenol caffeic acid affects life traits differently in the nematode *Caenorhabditis elegans* and the cladoceran *Moina macrocopa*. Fresenius Environ Bull 19, 1238–1244

Saul N, **Pietsch K**, Menzel R, Stürzenbaum SR, Steinberg CEW (**2010**) The Longevity Effect of Tannic Acid in *Caenorhabditis elegans*: Disposable Soma Meets Hormesis. J Gerontol A Biol Sci Med .Sci 65(6), 626-35

**Pietsch K**, Saul N, Menzel R, Stürzenbaum S, Steinberg CEW (**2009**) Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by *age-1*, *daf-2*, *sek-1*, and *unc-43*. Biogerontology 10(5), 565-578

Saul N, **Pietsch K**, Menzel R, Stürzenbaum SR, Steinberg CEW (**2009**) Catechin induced longevity in *C. elegans*: from key regulator genes to disposable soma. Mech Ageing Dev 130(8), 477-486

Saul N, **Pietsch K**, Menzel R, Steinberg CEW (**2008**) Quercetin-mediated longevity in *Caenorhabditis elegans*: Is DAF-16 involved? Mech Ageing Dev 129, 611-613

Steinberg CEW, Saul N, **Pietsch K**, Meinelt T, Rienau S, Menzel R (**2007**). Dissolved humic substances facilitate fish life in extreme aquatic environments and have the potential to extend lifespan of *Caenorhabditis elegans*. Annals of Environmental Science 1, 81-90

**Contributions to conferences**

**Pietsch K**, Stürzenbaum SR, Saul N, Menzel R, Steinberg CEW (**2009**) The physiological effects of polyphenol induced longevity. 17th International C. elegans Meeting in Los Angeles, POS 307 A

Saul N, **Pietsch K**, Menzel R, Steinberg CEW (**2009**) *C. elegans* and the Disposable Soma Theory: Extended lifespan and reduced body size due to exposure to tannic acid and catechin. 17th International *C. elegans* Meeting in Los Angeles, POS 308 B

Menzel R, **Pietsch K**, Saul N, Rienau S, Wild W, Staudacher H, Steinberg CEW (**2008**) Humic material induces behavioral and transcriptional responses in the nematode

*Caenorhabditis elegans* and extends its lifespan. European Worm Meeting in Sevilla, POS B-146

**Pietsch K**, Saul N, Menzel R, Stürzenbaum S, Steinberg CEW (**2008**) Effects of rosmarinic acid and (+/-)-catechin on *Caenorhabditis elegans* lifespan. European Worm Meeting in Sevilla, POS C-155

Saul N, **Pietsch K**, Menzel R, Steinberg CEW (**2008**) Analyzing quercetin mediated longevity in *C. elegans*. European Worm Meeting in Sevilla, Oral Presentation, T-14

**Pietsch K**, Saul N, Menzel R, Steinberg CEW (**2007**) Verlängerung der Lebensdauer durch das Polyphenol Quercetin im Nematoden *Caenorhabditis elegans*. Nachrichtenbl. Deut. Pflanzenschutzd. 59, 189

Saul N, **Pietsch K**, Menzel R, Steinberg CEW (**2007**) Quercetin Extends Lifespan in the Nematode *Caenorhabditis elegans.* Genetics of Aging, 39th Annual conference of the German Genetics Society (GfG) in Jena, POS 42

Steinberg CEW, Saul N, **Pietsch K**, Menzel R (**2007**) Lifespan extension in *Caenorhabditis elegans* by a model humic building block. 10th Humic Science & Technology Conference in Boston

Weitzel T, Collado A, **Pietsch K**, Jost A, Schmiedel C, Konjević A, Zgomba M, Petrić D, Foussadier R, Storch V, Becker N (**2006**) Biochemical differentiation of European *Culex* species. Society for Vector Ecology 37th Annual Meeting, Anchorage, Alaska. September 29th – October 3rd. Conference Program, 9: P-24

# Selbständigkeitserklärung

Hiermit erkläre ich, die Dissertation selbstständig und nur unter Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt zu haben.

Ich habe mich anderwärts nicht um einen Doktorgrad beworben und besitze einen entsprechenden Doktorgrad nicht.

Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät I der Humboldt-Universität zu Berlin.